

Microsoft's brush with regulators

The largest software manufacturer's brush with US and European antitrust regulators might have been avoided if copies of software were sold not as if they were books but as intellectual property more broadly.

THE truism of the computer era is that even the most sophisticated hardware is no better than the software with which it is equipped. That is almost a sufficient explanation of the dazzling rise of the company called Microsoft in just under two decades to become the largest software manufacturer in the world and one of the largest public corporations. Its big break came in 1978, when it was chosen to supply the operating system called MS-DOS for the personal computer made by IBM, which then put the world at Microsoft's feet not only by selling huge numbers of its own machines, but by the liberality with which it allowed them to be cloned. Since then, as the world knows, IBM has fallen on hard times. What will happen to Microsoft now that it has tangled with the US Department of Justice and the European Commission and has been forced to sign an agreement to avoid alleged anti-competitive practices for the next six and a half years?

It is too soon to tell. The unfair practices of which Microsoft was accused (and which the company says are false) are too arcane for their commercial effects to be guessed at. If, as has been alleged, Microsoft required computer manufacturers to whom MS-DOS had been licensed to pay a royalty on every microprocessor they sold, whether or not it was loaded with Microsoft's operating system, the consequence a decade ago may have been to dissuade manufacturers from installing other operating systems in their machines. But now, when the tide has rolled in Microsoft's direction, the cost of rolling it back again would far outstrip whatever efforts could be mounted by a couple of bright people working from a garage (the legend of the computer industry in the United States). If there is ever a serious push in that direction, it is more likely to come from a Japanese manufacturer than from within the United States, the virtues of the rival UNIX notwithstanding.

That is the sense in which Microsoft may be a victim of its own success. It has been technically ingenious, and it has differed from most large companies in avoiding the almost inescapable complacency to which the successful are especially prone. No sooner is one piece of software successfully launched than it is improved and then resold (with discounts for those who own the earlier versions). At no stage has Microsoft been accused of lethargy, or its products decried for poor quality. That it has grown huge and rich is, on the face of things, an accurate market evaluation of its competence.

But there is a flaw in that argument affecting not just Microsoft but the whole of the software business. It has to do

with copyright, and the terms on which computer programs should be sold to those who use them. The difficulty is easily illustrated (and commonly experienced). Suppose a person wishes to purchase a copy of his or her favourite word-processor called, say, Verbosity™ version 3.1. The chances are that the software store will explain that version 3.1 has long since been outdated, that version 8.3 is now in stock, and that it has many more bells and whistles than the older version. The price, the store will say, is 395 (in local units of currency). The would-be purchaser's protest that version 3.1 will suffice for the purposes intended is unlikely to unlock a legal copy of the older version from the stock-room.

The flaw in that argument, which also underlies the alleged causes of Microsoft's brush with the US Department of Justice, is that software manufacturers regard their products as successive editions of a book while knowing (as do their customers) that there is no resale market for the older editions, which are in any case often physically tied to the machines in which they are embodied. But if Verbosity™ 3.1 is no longer in print, natural justice (and patent, but not copyright, legislation) would require that others should be free to manufacture it. If some requirement of that kind were enforced, software manufacturers would quickly take to selling the bells and whistles separately from their core programmes. That is the direction in which the authorities should push the software manufacturers when they next have a chance to do so. □

Housekeeping at Oxford

Britain's oldest university will have to struggle hard to keep its independence.

READY access to higher education is a civilizing force and an economic necessity in states pretending to be sophisticated, but there is inevitably a danger that wider access brings uniformity. That, it seems, is part of the reason why the University of Oxford has embarked on another bout of introspection about its internal administration, this time by means of an internal inquiry under the vice-chancellor, Dr Peter North. By all accounts, no questions are off limits. Even the question of whether the university might turn itself into a private institution may get a hearing, if perhaps a cursory one.

The University of Oxford is the oldest in Britain, but that



is not the reason why it has a special claim on public attention. Nor, for that matter, is its tradition, which is often a spurious justification of irritating anachronism. What matters is the quality of the education offered to undergraduates, the imaginativeness of the training in research offered to graduate students, the range and depth of the university's scholarship and research and its value as an example of how a university should conduct its affairs. The North inquiry appears to have been prompted, nevertheless, mostly by external forces — notably by the continuing pressure on public support for British universities, with rules of thumb linking public subventions separately to student numbers and research attainment.

Oxford has a structural problem of its own choosing, the balance of power between the colleges at which students compete for places and to which they then nominally belong and the university which, in due course, awards the same students their degrees. At Oxford, the colleges collectively are relatively more powerful (because many of them are richer) than at the University of Cambridge, where in principle the same system obtains. It is not irrelevant that both systems have their roots in the mediaeval university, in which the university provided only public examinations while the colleges were dormitories at which students lived while being taught privately by tutors to whom they paid fees. That explains why at both universities (but with special insistence at Oxford), the provision of tutorial instruction by established academics is held to be a distinctive part of undergraduate education.

The tutorial system is indeed a valuable institution, but its value diminishes as the years go by, especially in science, where laboratory work and time spent in the computer room are ever more important. That does not imply that Oxford should be willing to abandon its tutorial system for the benefit of the university, but simply that its colleges should recognize the limitations of the system (not least, that while all tutors are supposed equal, some are more equal than others). There is a case for a decisive shift of responsibility for undergraduate education towards the central university. At a time when accountability is all the rage, students' accountability towards their teachers (by means of more regular examinations) would also serve a useful purpose.

But Oxford's most urgent need is to strengthen its research, which is patchy compared with that at Cambridge. To be sure, the two universities differ very little in the ratings their academic departments were awarded in last year's attempt at the assessment of research quality, but that is a misleading index. The Oxford problem, in any case, is not the absence of excellence, but the scope and depth of its collective research in science. The difficulty, in present circumstances, is that intensifying efforts across the board would require extra people (which would be expensive) and even bricks and mortar (which are even harder to come by now). At the very least, one outcome of the North inquiry should be a more effective mechanism for uniting the colleges and the university as a whole behind a coordinated strategy for research. □

Moon-landing blues

The 25 years since the first landing on the Moon have been a predictable disappointment for space flight.

THE celebrations last weekend of the anniversary of the departure of the Apollo XII spacecraft for the Moon could not have been timed better for those in the US Congress whose ambition is to see space-station Freedom well-funded and eventually launched into an orbit about the Earth. If the US National Aeronautics and Space Administration (NASA) had been hoping that the anniversary would rekindle taxpayers' enthusiasm for such adventures, it has probably not been disappointed. But suspicions that NASA arranged the impact of Shoemaker-Levy on Jupiter to enhance the general excitement are without foundation.

This anniversary (that of the 'walk' by Neil Armstrong and 'Buzz' Aldrin fell yesterday) shows how little has changed in a quarter of a century. In 1969, it seemed proper to praise both visitors to the Moon for their composure under trying circumstances (not the least of which must have been the international television audience of 600 million), to commend NASA for its management of the project, to remark that little would be discovered about the Moon that had not already been found out, to suggest that strictly scientific exploration of the rest of the Solar System would be better carried out by instrumented spacecraft, to call for a better way of deciding what space flight is for and to acknowledge that, nevertheless, the visit to the Moon fired the general imagination (see *Nature* 223, 333; 1969).

That opinion was over-confident of NASA's management, much clouded in recent years, and over-dismissive of the benefits that would accrue from the analysis of Moon rocks, which established that there were two distinct epochs of surface cratering early in the Moon's history, at about 4 billion years ago. The intellectual consequences of the landing on the Moon were also a surprise; people were more often persuaded by Apollo's startling images of the fragility of the Earth than that the next stopping-place must be Mars or somewhere like it.

The purpose of the Apollo venture was never simple. Decided on by President John F. Kennedy in the wake of the *Sputnik* humiliation, it was mostly a move in the Cold War. It restored the reputation of the United States for technological prowess, and it lifted American spirits to a greater degree than would have the distribution of the total cost (roughly \$20 billion) among the then-population (which would have given each \$100). But the purpose of space flight from the United States (or anywhere) is still undefined except in the language of the travel brochure, while the technological developments that might have made distant journeys feasible have not come about, largely through neglect. Meanwhile, in the United States, the space station project limps on from year to year (see page 167), exciting little enthusiasm or imagination. Would it not be better to concentrate instead of the exploration of the Solar System by the means already shown to be effective? □

Comet collision boosts controversy over global protection strategy

Washington. The greater than expected impact of the comet Shoemaker-Levy 9 as it crashed into Jupiter this week is set to intensify the debate over what steps, if any, should be taken to deal with the prospects of such an object striking the Earth.

Many astronomers are keen to proceed with an extensive survey of those comets and asteroids which are likely to infringe on the Earth's orbit, along the lines of the \$250 million Spaceguard proposal made two years ago by a panel set up by the National Aeronautics and Space Administration (NASA) — and as yet unfunded.

But some fear that the perceived threat is being overstated. In a strongly worded article in this summer's issue of the journal *Issues in Science and Technology*, Carl Sagan of Cornell University and Steven Ostro of NASA's Jet Propulsion Laboratory play down the risk.

Using a rough-and-ready risk analysis, they estimate that global impact catastrophes, when averaged out statistically, could account for 3,000 deaths per year. But they argue that this is insignificant when compared, for example, to the three million deaths a year from smoking, or those in developing countries dying from poverty and disease.

While supporting the Spaceguard proposal, Sagan and Ostro say they are alarmed at talk about the need for a nuclear weapons system to protect the planet from galactic debris. They warn that such a proposal is finding favour in "some elements of the defence establishment", even though such a weapons system is "well beyond the current economic capability of the planet, and would

introduce new risks that seem still more unacceptable".

The Spaceguard proposal would involve building and connecting six 2.5-metre diameter ground-based telescopes for \$50 million, and operating them at a cost of \$10 million a year for 20 years in order to survey asteroids crossing the Earth's trajectory and comets of more than one kilometre diameter.

Advocates say the survey would find 95 per cent of the asteroids, although they admit that its effectiveness in finding comets — which can arrive from anywhere in interstellar space — would be less easy to calculate. Some astronomers privately contend that the job could be done more cheaply with existing equipment.

Astronomers said this week that the Jupiter incident is likely to bring new impetus to the Spaceguard proposal. "It doesn't change the odds of a collision with Earth, but it makes the prospect more real," says Clark Chapman of the Planetary Science Institute, Tucson, Arizona. Paul Weissman of the Jet Propulsion Laboratory agrees. "It has demonstrated to people that comets do run into planets," he says.

The series of collisions with Jupiter — particularly that of the largest part, fragment H, which hit the planet on Monday after-

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

A series of infrared images in the K-band (2.2 μm), taken at one minute intervals on the evening of 16 July by the 0.75 metre telescope at the South African Astronomical Observatory, shows a flare caused by the first comet fragment (bottom left) increasing rapidly in brightness and then decaying slowly.

noon with a vast explosion — told astronomers at once that the density of the fragments was substantial, and that they had not disintegrated into a shoal of smaller meteorites as some had predicted.

Peter Leonard of the University of Maryland says that the density of comets had previously been estimated at between 0.2 and 0.9 that of water, the approximate densities of snow and ice respectively. But he estimated on Monday that the density of the fragment was around 0.5 — "like snow with dust in it, and well held together".

Several physicists said they were surprised at the scale of the observations they have been able to make, after many had predicted that the collision might not live up to earlier expectations.

David Crisp of the NASA Jet Propulsion Laboratory, the principal investigator of the team at the Anglo-Australian Observatory at the Siding Spring Observatory site in New South Wales, Australia, said the event had exceeded his expectations "many times over".

The team was viewing Jupiter with the observatory's 3.90-metre reflecting telescope using a near infrared camera, as the two sub-fragments of what was originally fragment G collided on Monday morning. According to Crisp, the smaller fragment produced a fairly weak flash, about twice as bright as those produced by fragments A and C. "Then at about 7.40 all hell broke loose when the larger fragment hit."

Colin Macilwain & Maggie Verrall

LHC delay hits Japan's physicists

Tokyo. Last month's failure by member states of the European Laboratory for Particle Physics (CERN) to agree on proceeding with plans for the Large Hadron Collider (LHC) has been a major setback for high-energy physicists in Japan who are trying to win their government's support for the proposed accelerator.

Japan's High Energy Committee, the top representative body of the high-energy physics community, decided late last year to throw its weight behind the LHC following the cancellation of the US Superconducting Super Collider (SSC) project. And the Ministry of Education, Science and Culture (MESC), which funds most of Japan's high-energy physics, is supporting the committee's position.

Officials from the ministry had been

looking for a way to respond to requests from CERN for Japanese financial support for the European laboratory (see *Nature* 361, 288; 1993). Anticipating a clear decision in Geneva, they had been planning to make a budget request to the Ministry of Finance for the LHC in their budget proposals for fiscal year 1995 (which begins on 1 April), to be submitted at the end of August.

But the failure of CERN's council to reach agreement means that "we missed the chance", says Hiroataka Sugawara, director general of Japan's National Laboratory for High Energy Physics (KEK). Official approval by CERN's council was "crucial" for MESC to win approval from the Finance Ministry, says Sugawara. "Now we will have to wait until at least next year".

David Swinbanks

Audit backs jailed professor's allegations

Quebec. Canada's largest research grant-making agency, the Natural Sciences and Engineering Research Council (NSERC), has frozen all grants to Concordia University in Montreal after an audit report showed serious irregularities in the university's engineering and computer science faculty.

Ironically, the contents of the report — as well as that of two others issued earlier this year — reinforce charges made by a former research professor in the faculty, Valery Fabrikant, who is currently serving a life sentence for murdering four of his colleagues in August 1992.

Fabrikant, who had been threatening and harassing faculty members for months before the murders, said he killed the four to draw attention to his charges of research fraud, contract irregularities, and what he claimed was an unjust denial of tenure.

Peter Morand, the president of the research council, says that in order to maintain its eligibility for future funds, the university must satisfy the council within 60 days that it has introduced mechanisms to correct the flaws that the report identifies. It must also return the misused funds.

The audit was carried out by the Montreal firm Bessner Gallay Schapira Kreisman, and revealed that research money had been put to unauthorized uses at the university for at least 20 years. It found that some faculty members, justifying their activities in the name of academic freedom, had carried out extensive freelance work.

Three engineering faculty members have had their research grants suspended and have been forced to leave the university. Seshadri Sankar will go on unpaid leave immediately before resigning on 31 December 1995. His brother Thiagas S. Sankar will take unpaid leave from July 28 until February, when he will begin early retirement. The third to leave is the former dean, M. N. S. Swamy, who will take early retirement on 1 September, when his current sabbatical leave ends.

An earlier report on the situation surrounding Fabrikant's violence against his academic colleagues was carried out by John Scott Cowan, former vice-rector of the University of Ottawa. It sharply criticized Concordia officials for allowing Fabrikant to carry on his vendetta.

Concordia's board of governors dismissed the rector, Patrick Kenntiff, a former law professor and Quebec civil servant, as well as three other senior staff immediately after the report was released on 31 May.

Correction: EMBL

The European Molecular Biology Laboratory's nucleotide sequence data base that is being transferred to its European Bioinformatics Institute in Cambridge covers sequences from all organisms, not just mammals as stated in our issue of 7 July.

A second report, prepared by a three-person committee chaired by Harry Arthurs, a former president of York University, compared Concordia's research culture to an automobile industry driven by quantity rather than quality. It said that "ends are sometimes used to justify means which are highly questionable", including "inappropriate authorial credit" and excessive outside work.

Fabrikant's output of 25 papers in less than four years was more than twice the average. On most of these Thiagas Sankar, the chairman of Fabrikant's department, was listed as co-author.

'More co-ordination needed' in EU

Schwerin, Germany. Paul Krüger, the German research minister, promised this week that Germany's presidency of the European Union (EU) will be marked by efforts to improve coordination between national and EU research programmes.

Krüger was speaking at an informal meeting of European research ministers held to mark the start of Germany's six-month presidency. Both he and the other ministers expressed in particular a desire to alter the role of the Science and Technology Research Committee (CREST), a body made up of two members from each country, and chaired by the European Commission (EC).

CREST was set up in 1974 to help promote community-wide research policy, partly by coordinating national policies with those of the European Communities. But many feel the committee has lost its original dynamism, while the recently appointed European Science and Technology Assembly has taken over much of its active advisory role.

The ministers discussed ways of improving CREST's effectiveness, for example by asking it to concentrate on longer-term EU research strategies and on the evaluation of past research policies.

Only 14 per cent of public research and development resources are spent through European-level organizations and agencies, and the EC Framework programme at present accounts for only 4 per cent. The rest of the money is provided through national programmes, which often do not cooperate with each other.

The EU research commissioner, Antonio Ruberti, says these figures show why closer coordination is essential. But coordination is a thorny problem, as it impinges on the right of national states to set their own research agendas, and conflicts with the principle of subsidiarity.

Nevertheless, there are signs that coordination is starting to enter the mentality of member states. For example, after meetings

The NSERC has now alleged that poor administrative practices allowed elaborate schemes to be set up in which false records were maintained, and the true nature of expenditures concealed both from the research councils and other funding bodies.

The NSERC's committee on professional and scientific misconduct will now consider whether further sanctions are warranted against the individuals involved. Charles Bertrand, the university's interim rector, has denied that the university let the three professors off lightly because of its own administrative failures. **David Spurgeon**

with the heads of national research councils in EU member states last month, research councils in Italy, France and Spain have presented two joint proposals to the commission concerning environmental research in the Mediterranean.

Ruberti is encouraging similar joint applications from other national research agencies, as well as the electrical and automobile industries. National research agencies will have to put forward a good proportion of the money for any participation. But Ruberti says he is prepared to provide a cash incentive — which he describes as a "wedding present" — for any coordinated project.

Other "wedding presents" are also under discussion. The seven leading aviation research establishments in Europe have combined to form an association which plans to develop common policy on cooperation among themselves and with the commission, and to share large-scale experimental and test facilities.

Krüger proposes that the commission should support this initiative by giving preferential support from its research programmes to research projects coordinated by the association.

The research ministers also discussed two other issues that have been concerning European researchers, namely why they should get such different rates of pay if they receive EC grants to work in other countries, and why applying for a grant is such a confusing and laborious task.

The EC's Human Capital and Mobility programme, which offers fellowships to young researchers to work in a different EU country, has been a major problem since its introduction early last year (see *Nature* **361**, 196; 1993).

The commission has set up a working group to try to solve this problem. But its task is likely to be difficult. "Ideally I would like to see no tax at all on these fellowships which should go entirely to research activities," says Ruberti. **Ailson Abbott**

Berlin universities head off major new cuts in funding

Munich. Fears of a massive cut in funds for Berlin's three universities, which threatened to close entire faculties, slash student numbers and cut staff numbers by about a thousand over the next eight years, have been partially averted following prolonged negotiations with the city's political representatives.

Cuts will still have to be made. But they will not be as extensive as the DM135 million (US\$85 million) proposed by the city's finance ministry in March. The universities are now working with politicians to identify how much money could reasonably be saved and exactly where the savings could be made.

But it will still not be easy to reach agreement on where the extra cuts should fall, even though this has to be achieved by the end



Erhardt: sees problems ahead.

of the year in order for the budget for the two years 1995 and 1996 to become effective.

The three universities — The Free University and the Technical University in the west, and the east's Humboldt University — each fear that any further cuts, coming directly on top of severe restructuring agreed last year, could cause significant damage.

All three agree that rationalization was essential after German unification in 1990. The Free University, for example, was established in 1949 to replace the Humboldt University, and there is now an expensive overlap of many faculties and departments in the university system of the reunited city.

Reunification also meant the loss of Berlin's generous subsidy from Bonn — for many decades, the West German government had supported the many cultural activities of the isolated city in order to make it a flagship of democracy.

At the end of last year, the painful process of defining a new, cheaper structure for the three universities appeared to have come to an end when a restructuring plan was approved by Berlin's parliament. This plan would have cut costs by DM130 million a year by 2003 — at present the annual bill for universities is around DM4 billion, including building and equipment — by reducing total student numbers by 15,000 to 100,000, with a corresponding reduction in staff. The reduction would be achieved primarily by merging departments and faculties.

In March, however, the universities were told by the city's finance ministry that they would have to reduce their costs by a further

DM135 million over the same period because of the city's unexpectedly severe financial crisis.

Manfred Erhardt, Berlin's research minister, tried to call the Berlin senate's bluff. He wrote to the senate explaining that such radical cuts could be achieved only by closing whole faculties — he mentioned biology and veterinary medicine — and reducing student numbers still further, possibly by an additional 10,000. He explained that he needed to be sure before he took any steps that the senate would take political responsibility for such drastic moves.

When the contents of the letter became public, there was uproar. As a result, the senate's finance committee offered a compromise, based on a proposal from a recently established Parliamentary working group comprising Erhardt and the science spokesmen from the two main coalition parties: CDU spokesman Eberhard Engler, from the Max Delbrück Centre for Molecular Medicine and SPD spokesman Bert Flemming, a physiologist from the Humboldt university.

Engler and Flemming had argued that a less adversarial mechanism should be set up to identify how cuts could be made. This would be made by a committee made up of four politicians (representing both science and finance committees), four representatives from the universities, and Erhardt.

Erhardt says he is not convinced that the committee will work because it is likely to find it difficult to reach a consensus. But he welcomed the decision that there will be no further reduction in student numbers.

The committee will discuss what further savings the universities can make that will be acceptable to all parties. But its decisions will not be made until autumn at the very earliest. Jealousies are being stirred up between the three competing universities. The Free University, for example, resents the large amounts of money spent on being spent on refurbishing the dilapidated Humboldt University. The Humboldt in turn defends its investment programme on the grounds that it needs to be able to compete with the Free University on equal terms.

Furthermore, all three universities are unhappy that they are being asked to bear the full burden of Berlin's cuts in its science budget, while non-university research establishments are being left untouched.

Erhardt claims that this is because Berlin pays the full cost of its universities, whereas the federal government pays a fixed proportion of the costs of the research establishment, and that this money would be lost to the city if it made its own support for these.

Alison Abbott

Space station critics vow to maintain fight against project

Washington. Opponents of the United States' international space station, having decisively failed in this year's attempt to kill the project in Congress (see *Nature* 370, 3; 1994), remain confident that it will succumb in 1996 or 1997, when the federal budget will be even tighter.

"This year's budget is going to look like a picnic compared with 1996, 1997 and 1998," says Tim Roemer (Democrat, Indiana) who moved the unsuccessful amendment in the House of Representatives to kill the station. There is a widespread feeling that a legally imposed cap on overall discretionary spending means that far larger cuts will be required in those years.

The relatively large amount of room for manoeuvre present in this year's budget was graphically demonstrated last week when the Senate budget bill, which includes spending by the National Aeronautics and Space Administration (NASA) and the National Science Foundation (NSF) was voted on in committee.

The Senate Veterans Affairs, Housing and Urban Development and independent agencies (VA-HUD) appropriations subcommittee, chaired by Barbara Mikulski (Democrat, Maryland), proposed budgets for NASA (\$14.4 billion) and NSF (\$3.45 billion) that are substantially above the president's requested level.

These budgets, which are likely to be approved by the full Senate, follow bartering between Mikulski and the White House, in the process of which she extracted an impressive list of concessions in exchange for her strong support of the space station.

The extra money has become available because the White House accepted adjustments in the housing budget that will relieve pressure on the rest of Mikulski's turf. The extra NSF money — \$300 million, compared to \$55 million requested — will be for construction of new laboratories and facilities, while NASA will get \$400 million that it had not requested to start building \$2.5 billion worth of wind tunnels.

The latter are immensely popular in Washington, as they are intended to help Boeing to compete with the European Airbus. Anyone suggesting that Boeing would be better off with a small wind tunnel and some computer simulation software does not understand how Congress works. Both Senate proposals have still to be agreed in conference with the House, but Senate staff say they now have the administration's support and are therefore likely to prevail.

Meanwhile Roemer is promising to subject NASA to ever-tighter scrutiny on the space station. He has inserted a provision in a House NASA authorization bill requiring ►

the agency to provide Congress with annual reports on how it is spending its space station money, and how the Russians are spending theirs.

"Dan Goldin [the administrator of NASA] has made some pretty grandiose promises which are going to be very difficult to live up to," says Roemer. "I'm going to hold him to these promises." He is also working with George Brown (Democrat, California), chairman of the House Science, Space and Technology Committee, on a provision in the bill that would cap annual station spending at its current level of \$2.1 billion.

Describing last month's vote in favour of the space station as "a temporary setback", Roemer concedes he made a tactical mistake in proposing that the station money go back into NASA, rather than making it a straight spending cut. "But I don't think it was one of the major reasons we lost," he says. "Probably in future, with the budget difficulties we face, a straight cut will be the way to go."

He does not see the 1995 vote as taking the project to the point where it cannot be cancelled. "We're talking about spending another \$50–\$55 billion on this," says Roemer. Very important projects such as Advanced X-Ray Astrophysics Facility, Cassini and Mars Surveyor may in fact be cancelled because of the space station. But for this year, as Mikulski's budget confirmed, these projects are safe.

Colin Macilwain

Warheads give boost to nuclear fuels

London. Uranium produced from the dismantling of nuclear warheads could provide up to 15 per cent of the world's supply of uranium fuel within ten years, according to forecasts made on behalf of the international uranium industry.

In a report published last week, the Uranium Institute, which is based in London, predicts that highly enriched uranium derived from obsolete nuclear weapons, and converted for use in commercial nuclear power plants, could be supplying between 5 and 15 per cent of world supplies over the next decade.

According to the institute, this supply will go some way to meeting the sharp rise in demand for uranium that it believes will take place in response to increasing global energy demands.

Under an arrangement reached with Russia in 1993, the United States has agreed to buy 500 tonnes of highly-enriched uranium resulting from the destruction of weapons. The destruction is itself one result of the START II treaty, signed by the then US president, George Bush, and his Russian counterpart, Boris Yeltsin, in 1993, in which both countries agreed to cut nuclear weapons by two-thirds.

Maggie Verrall

Locals seek strict controls over UCSF expansion plans

San Francisco. Plans by the space-starved University of California at San Francisco (UCSF) to build a new medical research complex on part of a former Army base in the city may have to meet stringent conditions laid down by local residents if they are to proceed with public support.

An alliance of nine neighbourhood groups is recommending several conditions to be attached to the UCSF plans for the 60-acre Letterman complex at the San Francisco Presidio site, which is due to become a national park this autumn. The conditions include a height limit on new buildings, measures to mitigate traffic, a promise not to expand beyond the federal property, ventilation systems and the proper disposal of hazardous materials — and an agreement to limit animals used in research to small rodents, such as mice and rats.

The site already houses a research centre and a hospital building. UCSF's planned medical research complex could cost between \$150 million and \$200 million for new construction and renovation, and would be used by 2,500–3,500 employees.

Research topics under consideration include population dynamics, epidemiology, microbial diseases, human genetics, structural biology, cell biology and other basic medical sciences. The university also plans to use the site for public education projects on biomedical research.

UCSF, which employs 15,000 people on four major sites, several smaller ones and in two affiliate hospitals, has had a tempestuous relationship with local residents in the recent past. Attempts to build research laboratories in a former insurance building in the prosperous Laurel Heights neighbourhood, for example, led to eight years of legal wrangling.

Now, as the university begins negotiations over potential leasing arrangements for the Presidio site, campus officials are determined not to repeat their mistakes. "We have learned from previous errors,"

says the vice chancellor, Bruce Spaulding, adding that the university has decided to involve local community groups in the planning process at a very early stage.

The university has recruited a group of 38 community advisers to help it to deal with local issues in developing plans for several potential sites for expansion. Many were selected because of their previous involvement in struggles with the university.

John Corsiglia, a member of the group and a neighbourhood activist who opposed the Laurel Heights expansion, calls the advisers "the best thing the university ever did". He says that since the committee was formed three years ago, the university had both listened and responded to those affected by its institutions.

In a plan to build a utility plant on its main campus earlier this year, for example, UCSF spent an additional \$4 million on soundproofing to meet neighbourhood concern about noise. "The university's image used to be 'we can do as we damn please' — and it was true," says Corsiglia. "That image is beginning to change."

In a bid to overcome hostility to the university's plans, UCSF officials have also held almost 20 workshops with neighbourhood associations and the general public to discuss potential locations. One suburb asked for help with local science education programmes, while an inner city neighbourhood sought assurances that local workers would get preference for construction jobs.

A primary concern behind all this has been the university's potential for unchecked growth through its use of state authority. Redmond Kernan, co-chair of the alliance, says many neighbours are concerned "because UCSF has a reputation for not being very easy to deal with". But he says the site might be a good one because the school would be kept in check by federal authority. "San Francisco needs to keep UCSF," Kernan says.

Sally Lehrman

Italy backs new national physics institute

Rome. The Italian government has approved a decision to transform the Interuniversity Consortium for the Physics of Matter, a body which links 36 Italian universities, into a National Institute for the Physics of Matter.

The consortium was set up in 1987 and is based in Genoa. It is responsible for a number of broad-ranging activities funded by both the Italian government and the EC, including programmes aimed at transferring technology to industry and at boosting the technology base in the

poorer regions of Italy.

The new institute, which will take over these responsibilities, will work closely with universities in fields such as condensed matter, quantum electronics, atomic molecular and plasma physics, computational physics, and biophysics.

It will have direct contacts with the European Synchrotron Radiation Facility in Grenoble and a domestic facility in Trieste. A key goal will be to promote the participation of Italian physicists in European research programmes. □

US physicists back linear collider proposal . . .

Washington. Ten months after the crushing blow delivered by the Congress's cancellation of the Superconducting Super Collider (SSC), US particle physics is getting off its knees. Its new plan is an old plan: for another massive collider, not so large as the SSC but not far off; not round, but straight; and not for protons, but for electrons.

Physicists are acutely aware of the public derision that may greet proposals for a new linear collider. Thus, when Sidney Drell, of the Stanford Linear Accelerator Center (SLAC), delivered his landmark report on the future of US high-energy physics in May (see *Nature* 369, 266; 1994), he emphasized immediate issues, and played down the section on future accelerator needs.

Furthermore, eyebrows were raised when Burt Richter, the director of SLAC, used a recent visit of the Emperor of Japan to advertise his proposal for a Next Linear Collider (NLC). Yet hope persists that, provided the painful lessons of the SSC debacle are properly learned, plans for such a linear collider can come to fruition.

The appropriate research, for example, needs to be completed beforehand, to enable project costs to be reliably fixed. The new machine also needs to be truly international from its inception. "This time, we're starting at the beginning," says SLAC's David Burke, who chairs a 23-strong international collaborative council set up last October to steer the project. "The SSC had severe problems trying to do it at the end."

The council includes representatives from eight establishments in the United States, ten in Western Europe, two in Russia and three in Asia. It will conduct formal reviews of the state of the embryonic project, the first being due for completion early next year. "The idea is to get everyone working on a common set of physics parameters for the machine," says Richter.

The machine would consist of two linear accelerators, one to drive electrons, the other positrons in the opposite direction. Physicists want collisions with an energy of 10^{12} TeV, which means 500 GeV in each direction. One way of achieving this would be to start by building two 250 GeV machines, and upgrade them later. In the case of SLAC's NLC design, the 250 GeV accelerators would each be 7.5 km long, with the potential of being upgraded either by doubling the length, or by doubling the acceleration rate.

One approach to the linear collider fa-



SLAC: looking to the future.

voured both by SLAC and by the Japanese national laboratory of high-energy physics, KEK (see box) would be a ramped-up version of SLAC's existing 50 GeV linear collider. But that technology is inefficient and the particle beams would have to be very finely focused.

Another approach being explored by the international Tesla collaboration, led by the German Deutsches Elektronen-Synchrotron (DESY) laboratory in Hamburg, is to use super-cooled, superconducting cavities to contain the beam. These are far more energy-efficient, thus removing the need to focus the beam so tightly. But they would be expensive, and require an even

longer accelerator to provide the necessary energy level.

Richter hopes design variants will all coalesce into a single internationally agreed design by 1997, with construction beginning in 1999. As he made clear during a visit to SLAC on 23 June by Emperor Akihito and Empress Michiko, Richter sees the construction of NLC primarily as a Pacific Rim project. "Europe is going to be tied up with the LHC through 2003 or 2005," says Richter. "If we want to start before then, the major players are going to have to be non-European." But Japan has its own plans for a collider, and is unlikely to support one built on US soil. The US Congress is also likely to be baffled by calls for a big straight collider in place of the circular one it has only recently abandoned.

But from the particle physicists' point of view, electron colliders and proton rings complement each other. For example, a proton ring at Fermilab recently came close to finding the mass of the top quark; armed with this information, an electron collider will be more effective in producing large numbers of top quarks for further study.

Research work is proceeding on the linear accelerator design and the US Department of Energy, as well as other funding sources around the world, appears happy to pay for the early research.

Funding the project itself will be more problematic. Richter says it is too early to estimate the total cost. But the engineering task is around one-third that of the SSC, which was due to cost around \$11 billion at the time of its death.

Colin Macilwain

. . . but Japan wants to take the lead

Tokyo. Japan's high-energy physicists, who are developing their own plans for a giant linear collider, say that it is highly unlikely that their government would contribute to a collider built in the United States.

The Japan Linear Collider (JLC) would consist of two linacs with a combined total length of 25 kilometres. In the first phase, the energy would be set at 150 GeV per linac, and this would be upgraded to 250 GeV each within a few years (see *Nature* 358, 266; 1992).

The project has not yet received official backing from the Japanese government. But considerable progress has been made on research and development for the collider using general research funds. Next year, for example, the National Laboratory for High Energy Physics (KEK) in Tsukuba will open a facility for testing components for the collider.

KEK is collaborating with the Stanford Linear Accelerator Center (SLAC) in the United States in this initial phase, and researchers from the two institutions recently succeeded in producing a 74 nanometre beam spot at SLAC. A critical

requirement for the JLC design is a beam at the collision point which is only a few nanometres in height and a few hundred nanometres in width.

But Japanese high-energy physicists are determined to take the leading role in building the world's next linear collider. Given their bitter experiences over the Superconducting Super Collider (SSC) where pressure was repeatedly put on Japan to support SSC construction costs until the project was finally killed by the US Congress, they have no intention of taking a supporting role in a US project.

"We do not want to repeat the mistakes of the SSC," says Hirofusa Sugawara, director general of KEK. The JLC was adopted as a future domestic project by Japan's high-energy physics community in 1986, and scientists are keen on international participation. "But we can't expect large contributions [from overseas]," says Sugawara. "We don't have politicians like [George] Bush to push for us" — a reference to the former president's role in promoting the SSC.

David Swinbanks

Peer review: NIH urged to streamline bids ...

Washington. Senior scientists in the US biomedical research community last week expressed enthusiasm for simplifying the processing of grant applications by the National Institutes of Health (NIH) by using only outline proposals for initial screening.

Many agreed that peer review is now too nit-picking and needs to get back to the main purpose of evaluating scientific merit. The favoured approach is being referred to as 'just in time', after the concept developed by the Japanese car industry. There was less enthusiasm for a proposal set-price grants for small projects.

But Bruce Alberts, president of the National Academy of Sciences, also admitted that "things can go wrong" with the scientific panels that are central to the NIH's system of peer review.

Alberts was speaking at a meeting organized by Harold Varmus, director of the NIH, to discuss ways in which peer review could be made fairer, more efficient and less cumbersome for both applicant and reviewer.

The NIH has a two-tiered process for evaluating unsolicited proposals. Initially such proposals go to a panel of scientists, known as a study section, who have expertise in a particular field — such as biomedical endocrinology — and review applications for their scientific merit. An application receiving a sufficiently high score then passes to a second panel within the relevant institute. This decides whether the proposal fits the institute's research priorities.

The NIH is already experimenting with one modification intended to reduce the workload of the study sections (see *Nature* 369, 269; 1994). But many feel that more sweeping changes are needed. As funding has become tighter in recent years, for example, applicants have refined the art of grantsmanship, fine-tuning their applications to meet anticipated questions.

Aubert appointed as director of CNRS

Paris. Guy Aubert, currently director of the Ecole Normale Supérieure in Lyons, has been officially nominated to succeed François Kourilsky as director-general of France's Centre National de la Recherche Scientifique.

Aubert is a physicist specializing in magnetic materials, and has been director of the Lyons institution, which he helped to create in 1985, since 1988. He was also rapporteur for the series of colloquia on research organized last year by François Fillon, the research minister, and was responsible for preparing the "synthesis" report submitted to the final national colloquium earlier this year. □

At the same time, in an attempt to make fine distinctions among many high-quality proposals, study section members can find themselves discussing the minutiae of experimental methods. "My concern is to get back to a time when the only thing that a study section considered was scientific merit," says David Botstein, chair of the department of genetics at Stanford University.

The budgetary squeeze that is largely responsible for the close scrutiny is unlikely to go away in the near future, so the NIH is looking for organizational changes that would throw the emphasis of review back to a consideration of overall scientific merit.

The 'just in time' idea met with general approval. Researchers would submit an application detailing the science proposed but containing only a rough outline of costs; a detailed budget would be submitted only if the study section approved the science. "This could do for the NIH and the universities what it did for Toyota," said Botstein.

Other suggestions were more controver-

sial. One was a proposal that scientists in search of smaller grants might apply for preset amounts, say \$100,000 or \$150,000. This would free the applicant from having to account for every cent spent on equipment, allowing the study section to concentrate on the quality of science, and whether it could be done for the sum requested.

But despite favourable comments — and the fact that a National Commission on Research recommended this approach in 1980 — several participants voiced concern over how the amounts would be set, and what would happen if the price of a piece of research fell between two preset levels.

A less popular suggestion for reducing the time spent on reviewing applications was that scientists with an established track record should be allowed to write a shorter proposal than junior scientists. Sharon Murphy, chief of haematology and oncology at the Children's Memorial Hospital in Chicago, said it might be seen as an "old fogey's network".

The composition and organization of the ►

... as Britain seeks to reassure

London. Britain's Office of Science and Technology has launched what one observer describes as a "charm offensive" designed to reassure university researchers that new procedures for evaluating research grant applications will continue to make significant use of the peer-review process.

Last week, William Waldegrave, the minister for science (and rumoured at the time as a potential victim in this week's anticipated cabinet reshuffle), issued a statement in which he confirmed that peer review would "remain centre stage".

The statement was intended to reassure parts of the scientific community — whose concern has already been picked up by opposition politicians — about the implications of new procedures for evaluating research grant applications being introduced by, in particular, the Engineering and Physical Sciences Research Council (EPSRC).

In particular, fears have been expressed that the explicit mission given to the research councils in last year's white paper of contributing towards wealth creation could reduce the emphasis placed on scientific quality in assessing whether a particular application is funded.

There is also concern that the introduction of non-scientific criteria in allocating research funds — together with a decision by the research councils to streamline the functions of their scientific advisory committees and place greater responsibility in the hands of programme managers — will reduce the influence of the scientific com-

munity on the councils' decisions (see *Nature* 368, 85; & 369, 3; 1994).

In defending the new system after its formal approval by the council last month, Richard Brook, professor of materials at the University of Oxford and the newly appointed chief executive officer of the EPSRC, claimed that his goal is to improve the efficiency with which grant applications are processed.

Most scientists seem to approve of the steps to streamline peer review, agreeing

that the present system is both cumbersome and time-consuming. Until now, for example, all applications have been considered by a full peer-review committee; future applications will receive an initial screening by three individuals, and only those passing this hurdle will be considered by a full review group, made up of individuals selected from a pool of members of a subject-based 'college'.

Grant-holders say that they are also reassured by the Office of Science and Technology's insistence that council officials, in deciding which applications are to be funded, "will not vary the rankings of scientific quality made by the peer reviewers". ►

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Brook: seeking
greater efficiency.



study sections also received close attention at the meeting. Alberts lent the weight of his office to the debate by pointing out that biology is changing more rapidly than the composition of the study sections. "Even when the study sections reflect the current fields in biology, their expertise decays and they cannot attract the likes of a Harold Varmus," he said.

His remarks stimulated nods of agreement. Ira Mellman, a professor in the department of cell biology at Yale University said that the composition of the study sections was the most important public policy decision facing NIH over how to spend its money. Asked later if he agreed with Mellman's assessment, Alberts said: "Yes, I like that."

The issue is to be addressed by Keith Yamamoto, chair of the department of pharmacology at the University of California, San Francisco, at a meeting in the autumn. Yamamoto points out that work on hormone receptors would once have involved only physiologists, but now it involves X-ray crystallographers, geneticists and developmental biologists.

NIH admits that it can be difficult to recruit people to study sections. Appointments last for four years, and are time-consuming. The feeling at last week's meeting was that peer review carries the same civic responsibility as jury duty. Dissatisfaction was expressed with scientists who have received grants from the NIH yet refuse to serve as peer reviewers. Many can expect a personal phone call from Varmus during the coming months. **Helen Gavaghan**

World Bank report slams Western-style university model

London. The European model of higher education is inefficient, relies too heavily on government funding, and is inappropriate for developing countries, according to a World Bank study published last week.

Faced with a worldwide increase in demand, countries are having to maintain or improve standards of higher education at the same time as budgets are being cut. The crisis has been most acute in developing countries, says the report, where expanding student numbers have had a dramatic impact.

A contraction in student expenditure — in Sub-Saharan Africa, for example, this fell from an average of \$6,300 per student in 1980 to \$1,500 in 1988 — has meant that the quality of teaching and research in many countries has deteriorated "precipitously" says the report.

Higher education institutions in these countries are faced with overcrowding, deteriorating physical facilities, and lack of resources for textbooks, educational materials and basic laboratory consumables.

The report says that science and technology has been particularly badly affected in developing countries. This is reflected in the fall in scientific output. In Ghana, for example, the number of science papers published dropped by 67 per cent between 1977 and 1987; there was a decline of 53 per cent in Uganda over the same period.

The World Bank, which has lent US\$5.1 billion for higher education since 1980, says that the solution lies in greater private financing of higher education, accompanied by improved efficiency and quality in publicly-funded institutes.

In particular, it wants reform to move in four key directions: encouraging a greater differentiation of institutions (including the development of private institutions); giving public institutions incentives to explore alternative sources of funding; redefining the role of the government; and introducing policies to give priority to quality and equity.

According to Thomas Eisemon, one of the report's authors and a senior specialist at the World Bank's education department, the decline in science research output, particularly in Africa, is the natural consequence of lack of resources. In some countries lecturers are having to supplement salaries as low as US\$30 per month by taking on additional jobs, while the buildings themselves fall into disrepair. As he says: "You can't teach biochemistry under a tree."

The controversial findings of the report echo feelings in post-communist east central Europe that countries should not emulate western university systems, but should instead learn by their mistakes (see *Nature* 369, 600; 1994). **Maggie Verrall**

doubters over policy changes

But even with such reassurance, there remains concern that a commitment to pursue wealth creation will inevitably mean that, whatever peer-review judgement is made, the final decision on a particular grant application will also involve non-scientific criteria. According to the council, for example, even in the pre-screening of research applications, one of the three reviews will be expected to reflect the views of the potential 'users' of the results of the research.

More generally, there is unease among some scientists that the abolition of the council's scientific committees will deprive those engaged in the peer-review process of a chance to develop a proper overview of their discipline. "Peer review is not the centre of our concerns," says John Ringrose of the University of Newcastle upon Tyne, who is president of the London Mathematical Society, Britain's main body for professional mathematicians. "What worries us is that the EPSRC may adopt an approach to research and research training that takes little account of those involved in the peer review process."

According to Ringrose, for example, the only "coherent view" of a field will now be that held by programme managers. He and others suggest that this will inevitably shift the balance of power between the council (and its officials) and the scientific community when it comes to strategic decisions.

Council officials defend the new changes as part of their efforts to increase the efficiency with which research grants are han-

dled. While playing down the extent to which the council has been motivated by government directives to reduce costs, one motivation has been the need to reduce administrative costs.

Brook denies that scientists have been deprived of an opportunity to inject their ideas into the policy-formulating process. He points out that this will now be done through the new 'technical opportunities panel' which, in parallel to a separate 'users panel', is being set up to advise the council on its strategic priorities.

Senior officials of all six research councils last week had a meeting with officers of the Royal Society in London, which had expressed concern at the potential threat to the tradition of reviewing applications purely on the basis of scientific excellence.

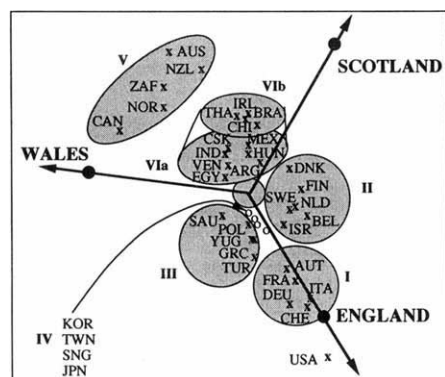
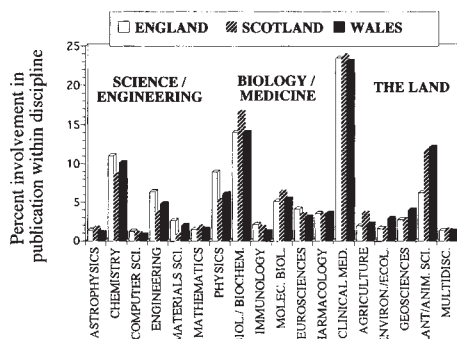
After the meeting, Sir Michael Atiyah, the president of the Royal Society, said that he had been "moderately assured". But he added that he was reserving final judgement until further details have been published of precisely how the new mechanisms are to be put into practice.

The EPSRC is planning to circulate its detailed proposals on issues such as the planned arrangements of the discipline-based 'colleges' soon, with a goal of introducing the new procedures on 1 January next year. Both Brook and Alan Rudge, chairman of the EPSRC, will discuss the proposals at open meetings throughout the country in September and October. These promise to be lively affairs. **David Dickson**

Regional Cinderellas

SIR — Recent articles (*Nature* 368, 379 & 383; 1994) emphasized how regions like Wales and some member states of the European Union (EU) might feel neglected when it came to allocation of funds by central authorities. In this respect, a confrontation with scientific output as measured by publication of mainstream research (data for 1981–92 from the Institute for Scientific Information) is enlightening.

The public financial input into Wales (3.7 per cent of total research council expenditure) correlates well with its scientific



output (about 3.8 per cent of UK publications). Its publication pattern (top panel of figure) shows that, like Scotland, it focuses on disciplines connected with the land. In Wales, publication on ecological and environmental issues is comparatively high and probably linked to high National Environmental Research Council spending. Scotland receives more funds from the Medical Research Council and publishes preferentially in the life sciences as is expected of centres of medical excellence.

The publication pattern of a nation is tantamount to a signature. It reflects a nation's interest in a discipline and its capacity to perform mainstream research

in human, material, and financial terms. The publication patterns of 45 nations have been compared with those of the UK triad (>6 million references) by Correspondence Factor Analysis¹ (bottom panel of figure). Countries with similar signatures are close; countries analogous to a UK region are furthest along the vector linking the origin to this region.

England is a more powerful vector than Scotland or Wales and strongly associated with the United States. Do these two countries advance on every scientific front and hold the reins of upper-echelon journals in all disciplines? The countries closest to England that have opted for a similar balance of priorities are mostly EU members (I). On the Scottish side of the 'England' vector lie Israel, the Benelux, and Scandinavia (excluding Norway) (II). Scandinavia shares an interest with Scotland in life sciences. On the Welsh side are part of the Mediterranean rim, Poland and Saudi Arabia (III). Four Asian countries (Japan, Singapore, Taiwan, South Korea) (IV) are well lined along the vector leading to England and the United States.

Facing England, somewhat afield and nestled between Wales and Scotland, is a zone of several Commonwealth countries (V). The UK regions and the Commonwealth may be linked not only by British tradition but by a common heed for the land as Norway, highly mindful of its natural resources, belongs to the group. Also facing England, but more central, are India, Egypt, the former East European bloc, as well as Mexico, Argentina, and Venezuela (VIa). An adjacent small group (VIb), nearer to Scotland, includes Ireland, Brazil and Chile.

Taken together, these results suggest to us that culture, as forged both by geographic proximity and historical influence, strongly influences the pattern of scientific output by nations largely regardless of scientific merit. A preliminary time-analysis seems to indicate a trend toward conformism in Western countries, which, if corroborated, might justify giving some 'regional cinderellas' more autonomy in selecting their own research policies.

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Mars mission

SIR — Tony Reichardt is correct in reporting (*Nature* 369, 3; 1994) that the Russian-led mission Mars 94 "has fallen victim to financial, technical and scheduling problems" and that the launch has been delayed until 1996. The problems are well known and are due to the difficult financial and political circumstances of our Russian colleagues. The delay in the launch date of Mars 94 has been widely anticipated for many months. Nevertheless, it is a great tribute to the initiative and tenacity of the Russian Mars 94 scientists and engineers that the mission is still viable.

We would like to make one correction. You report that there were technical delays with the "German-built Argus platform for pointing the spacecraft's cameras". In fact Germany has never had any involvement in the construction of the platform, which, like the spacecraft itself, is a Russian responsibility. However, since the platform is critical also for a successful mission in 1996, discussions are taking place between the Russian, German and French sides to identify areas in which assistance could be provided to our Russian partners. Completion of the platform on schedule is crucial to the effective operation of the German and French instruments to be mounted upon it.

Gerhard Neukum

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Vatican error

SIR — Writing about the Pontifical Academy for Life (*Nature* 369, 352; 1994), Gerard LeBlond justifiably found fault with the sentence "life begins at the moment of conception". Of course the sentence assumes that life exists in the germ line before, during and after the moment of conception and that life goes on throughout the development of an organism; the statement, as LeBlond probably comprehends, was made to underline the beginning of a new individual, different from its parents, and this can occur only at the moment of conception when the genetic material of two germ cells contributes to create a different organism.

This point of view supports the development of a wide defence of unborn rights and justifies the great effort to create an Academy for Life which guarantees human rights from this mysterious moment onwards.

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Russia's new science survivors

The survival of a core of the Russian research enterprise seems more certain now than for several months. That prospect is the achievement of the research minister for the past three years.

Moscow. Boris G. Saltykov, Russia's minister for research, was in a cheerful mood a week ago: he had been travelling about, talking to researchers and visiting some of the sites at which, even now, there are funds to support new developments. He was especially proud that the Russian particle-physics community (in partnership with Germany's DESY laboratory at Hamburg) has thought of using the great depth of Lake Baikal to bury a neutrino-detector beneath 1,000 metres of the world's most pristine water (now being shipped onto the international table-water market in plastic bottles). Construction is well under way. The implication is that Russian science will survive.

If true, that will owe as much to Saltykov and to his survival so far as to any other circumstance. Indeed, last week he wryly pointed out that, in a news report of the "special" (and really quite extraordinary) meeting of the old Soviet Academy of Sciences at which it was decided that the academy would become the Russian Academy of Sciences and at which Saltykov made his first public appearance as Minister for Higher Education and Research, this journal reported the views of some who held that he was "too intelligent to survive". "But look," he said last week, "I'm still here."

It has not always been like this. On a visit to London at the end of 1992, and with the trouble between President Boris Yeltsin and the parliament (*Duma*) reaching one of several climaxes, Saltykov confessed one evening that he did not know what job, if any, he would return to on the following day's aircraft. What happened was simply that responsibility for higher education was reassigned to another minister.

So Saltykov has good reason to feel secure. His intelligence has proved an asset, not a handicap. His well-mannered modesty works in the same direction. And whoever, in previous regimes, heard of a minister who visited dependent researchers in their own laboratories or in the field, when they could more conveniently be summoned to Moscow to make a presentation of their work?

Not that life is trouble-free. There is, for example, the endless problem of money. Half-way through the financial year, it is still not clear how much money will materialize for the second half. The only certainty is that it will be insufficient. Saltykov is not surprised that a number of research institutes have been compelled to give their staffs unpaid leave during the long (but unusually cool) summer.

Indeed, the ministry seems to be willing to

superintend a system of survival by natural selection. The institutes complaining that they cannot recruit graduate students, and which therefore worry about their future, are simply not paying them well enough. The institutes have to increase their income somehow. Successful institutes manage well



Saltykov is still there

enough, and their senior people are in good shape, earning between 200,000 and 500,000 rubles (the equivalent of \$100 to \$250) a month, perhaps with a spell of three to four months overseas each year.

Saltykov last week cited what seems to him a case of successful research entrepreneurship. A group of laser physicists at St Petersburg, having failed to win extra resources from the Physical Technical Institute, moved out to found their own laboratory and are now doing well, partly with the help of research contracts from abroad. If to survive is to live well, wit is a precondition.

Whether high-energy physics will manage to survive in Russia is another question. Saltykov says "I'm glad it's not my problem", but the accelerator laboratory at Serpukhov is now jointly financed by the ministries for atomic energy and for research. Construction of a 400 GeV proton accelerator was well under way eight years ago (see *Nature* **329**, 789; 1987), but even the 'warm magnet' accelerator, intended to produce protons at 400 GeV, is still two or three years from completion. Ambitions to

install superconducting magnets in the same 27-km tunnel to produce protons at 3 TeV are in abeyance. Inevitably, it has become a live issue to decide whether the installation should be finished. Saltykov says he does not know how the question will be decided, but notes that Russian scientists have access to CERN at Geneva and DESY as well as to Russian machines at St Petersburg and Novosibirsk. Saltykov is much more secure than Serpukhov.

On relations with the Russian academy, Saltykov said last week that "we're now in a calm period", meaning that the academy, while still asserting its right of access to the prime minister directly, is willing to talk to his own ministry as well. He hopes to build on that by inviting the academy's advice on science policy issues. As the academy's direct influence on research declines with its declining share of most institutes' budgets, that could be a constructive development.

Otherwise, Saltykov's chief concern is with the need to break new ground in the development of previously neglected institutions. Industrial research is still mostly concentrated in ministry (and academy) institutes; there is no immediate prospect that even newly privatized industries will set up their own research facilities. But that, Saltykov says, is a measure of the degree to which direct foreign investment in Russia has fallen behind the promises of the past few years. With the exception of George Soros's "excellent example of direct investment in Russian science", he said last week, "I'm still waiting to see it".

On the future of Russian universities, Saltykov is also sanguine. In language that might be heard from almost any education minister in the West, he notes that there are perhaps a dozen universities of the first rank among Russia's eighty, but again it is plain that their future as centres of research will hang on their people's flair for raising funds, perhaps from the Soros funds or perhaps from the government's own research foundation. In such hard times, there is no choice.

In all the circumstances, Saltykov is entirely right to be pleased with himself, and with his own survival in his post. He took office when rubles were worth 2,000 times what they are at present. The government has been through a succession of crises, including one in which it called out the tanks to shell the *Duma*. But now, despite the persisting problems, he seems to have assured the future of at least a core of Russian science. Perhaps the intelligence was a help, after all.

John Maddox

X-rays strike p53 again

Bert Vogelstein and Kenneth W. Kinzler

ONE way in which the tumour-suppressor protein p53 seems to suppress tumour growth is to permit apoptosis in cells that have become tumorous. This function was observed in cells induced to undergo

of a large β -sandwich that acts as a scaffold for three loop-based elements (Fig. 2):

■ The sandwich is made up of two anti-parallel β -sheets containing four and five β -strands, respectively. The scaffold

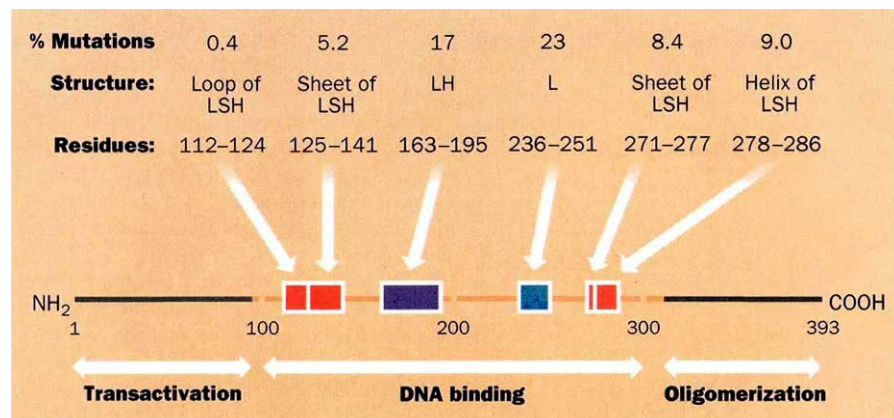


FIG. 1 p53 functional domains. The percentage of total p53 mutations located within the indicated regions is taken from data compiled by Bennett, Harris and colleagues¹⁵, which include over 2,600 mutations from human tumours. The orange line corresponds to the core domain used for crystallization. Colour coding is the same for Figs 1 and 2.

apoptosis by X-irradiation, and X-rays continue to play a pivotal role in p53 research. In last week's issue of *Science*¹, Cho *et al.* unveiled the X-ray diffraction structure of the core domain of p53 bound to DNA, and on page 220 of this issue² Caelles *et al.* use X-irradiation to probe p53 function in apoptosis.

The realization that p53 is mutated in a wide variety of human cancers has stimulated an intensive examination of its properties³. p53 can bind to specific DNA sequences of the general form (PuPuPuC(A/T)(A/T)GPyPyPy)₂. The central region of p53 (comprising amino-acid residues from about 100 to 300) contains the DNA-binding (core) domain. This proteolysis-resistant core is flanked by a carboxy-terminal region mediating oligomerization and an amino-terminal region containing a strong transcription activation signal (Fig. 1). One model for p53 function thus involves the sequence-specific transactivation of target genes. Candidate cellular targets have been identified that can inhibit cell growth and are regulated by p53. Importantly, the p53 mutants found in tumours are uniformly defective in sequence-specific transactivation⁴.

Cho and colleagues have now succeeded in co-crystallizing the core domain of p53 bound to DNA¹, and nothing quite like it has been seen before. p53 is devoid of the structural elements found in other DNA-binding proteins (such as zinc-fingers or the helix-loop-helix motif). Instead, its structure is unique, consisting

anchors the loops and participates in head-to-tail dimerization.

■ The first loop (LSH for loop-sheet-helix) binds to DNA within the major groove.

■ The second loop (L) binds to DNA within the minor groove.

■ The third loop (LH for loop-helix) packs against L and stabilizes it. The L and LH loops are held together, in part, by a tetrahedrally coordinated zinc atom.

One of the most notable features of the structure is its correlation with the data on mutations. The residues most frequently mutated in cancers are all at or near the protein-DNA interface, far from the hydrophobic centre of the β -sandwich. Over two-thirds of the missense mutations so far recorded are in loops L, LH or LSH (Fig. 1). The structure predicts two functional classes of mutations¹. Class I affects residues that directly contact DNA. These include Arg 248 and Arg 273, which are the most frequently mutated residues in p53. Class II mutants affect residues that do not contact DNA directly but which make extensive contacts with other residues in the polypeptide and have a critical role in stabilizing protein structure. For

example, class II mutants include those with a carcinogen-associated mutation at Arg 249 and those having the common mutation at Arg 175, both of which connect the L and LH loops (Fig. 2).

Previous biochemical experiments nicely support this mutant classification. Class II mutants seem to unfold the protein substantially, making p53 accessible to antibody 240, which binds to residues deep within the hydrophobic centre of the β -sandwich (residues 213-217; ref. 5). Class I mutants do not bind to this antibody unless the proteins are first denatured. Class II mutants are also more susceptible to proteolysis than class I mutants or native p53, consistent with their more open, unfolded conformation.

The predicted structure raises several questions. The first question, generic to models based on crystals, is whether the observed structure reflects that *in vivo*. In particular, it might be argued that the interaction between p53 monomers as observed in the crystal is an artefact of the co-crystallization process. Several previous studies have shown that p53-p53 interactions in solution are mediated through the carboxy terminus^{6,7}, a region absent from the core domain of p53 used for crystallization. The role of the carboxy-terminal region in mediating tetramerization is strongly supported by NMR spectroscopy⁸. Recent studies, however, show that the core domain of p53 can also form dimers and higher multimers in solution⁹. The crystal structure suggests that pairs of dimers might interact through the helices within the LH loops, perhaps facilitating a more efficient tetramerization by the carboxy-terminal domains present in the native protein.

A second, related question concerns the structure of this tetramer, which is predicted to bind to four copies of the half-

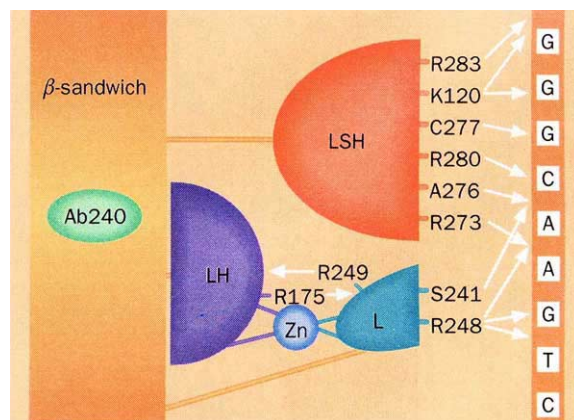


FIG. 2 Key structural elements of p53. Arrows connect relevant amino acids (single-letter code) in p53 to the consensus DNA sequences they contact. For simplicity, only one of the two strands of DNA is shown, though the contacts occur on both strands. Arrows pointing to bases represent contacts, while those pointing to the spaces between bases represent contacts to sugar or phosphate groups. Arg 248 contacts the indicated G through an H₂O bridge. Some of the residues connecting the L and LH loops are also shown.

site PuPuPuC(A/T). The way in which the DNA-binding dimer observed in the crystal relates to the tetramer is not clear, although a model has been constructed¹. A third question revolves around the incredibly diverse set of p53 mutations found in tumours. Although the most common mutations can be explained as described above, there are many rare mutations that also seem to inactivate the protein and are distributed over 125 different amino acids within the core domain. Nothing in the X-ray structure unambiguously reveals why so many residues in p53 can have such a drastic effect on function, or why (and indeed, whether) p53 amino acids are more functionally 'interconnected' than those in other DNA-binding proteins.

Cho and colleagues conclude their article with the statement that the crystal structure "solidifies the hypothesis that DNA binding and transactivation are the critical activities required for tumour suppression". The beauty and simplicity of this hypothesis, however, is challenged by the paper from Caelles *et al.*². The normal p53 gene suppresses tumour growth by causing cells to arrest in the G1 phase of the cell cycle or by causing them to become so disgruntled with their physiological state that they commit suicide (apoptosis)¹⁰. Caelles *et al.* immortalized pituitary cells with the simian virus SV40 T-antigen and then transferred a temperature-sensitive p53 gene into the cells. Following irradiation, the cells became apoptotic only when the wild-type p53 gene product was expressed at the permissive temperature. What was novel about this p53-dependent apoptosis was that it was not inhibited by hefty doses of transcription or translation inhibitors. Of course, it is impossible to exclude the possibility that some genes escaped the inhibition. In this regard, it would be interesting to examine the p53-dependent expression of Bax, a Bcl-2-binding and apoptosis-inducing gene product¹¹. However, the most straightforward interpretation of the data is that p53-induced

apoptosis in this system was not brought about by the sequence-specific transactivation of genes, but was the result of some other functional property of p53 (ref. 2).

How do these results compare with others? One of the most important of the systems relating p53 to apoptosis involves p53 'knockout' mice^{12,13}. Thymocytes from these mice are resistant to apoptosis upon X-irradiation, unlike those of normal mice. Transcription and translation inhibitors block p53-dependent apoptosis in thymocytes¹⁴, unlike the situation in the pituitary cells. It is possible, as suggested by Caelles *et al.*, that some of the effects of p53 (for example, G1 arrest) may be related to sequence-specific transactivation while others (apoptosis, at least in certain contexts) may be dependent on other actions of p53. Perhaps the unusual structure of p53 is telling us that p53 does something important besides activating expression of target genes. The results of Caelles *et al.* should provoke a careful re-examination of the necessity for transcription and translation in p53-mediated phenomena, and a renewed search for the

critical biochemical properties of p53 in situations where specific gene activation is not involved.

Whatever the normal function(s) of p53 in growth control, it is clear that the mutants have lost them (or they would not have been selected for *in vivo*). One practical goal of p53 research is therefore to convert mutant p53 proteins to wild-type forms. The structure of p53 provides an entirely new and powerful step towards this goal¹. Can small molecules be found, for example, that link the L and LH loops of p53 in proteins containing an Arg 175 mutation? How about a drug that allows a mutant of Arg 273 to bind to the phosphate that the wild-type amino acid normally contacts? The design of such 'restoring' compounds is no mean feat, but it is likely that computer modelling programs in both industry and academia will soon be humming the same X-ray-stimulated tune. □

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CLIMATE

Maize suffers a sea-change

Cynthia Rosenzweig

THE paper by Cane, Eshel and Buckland¹ on page 204 of this issue marks a sea-change in climate forecasting on a global scale. Indices of sea surface temperatures in the eastern equatorial Pacific Ocean are, they find, remarkably highly correlated with maize yields in Zimbabwe, on the other side of the world. Intriguingly, these correlations exceed those between the same indices and annual precipitation. The forecasts can be made far enough in advance to be useful in agricultural management. Foreknowledge of drought — in advance of sowing — allows farmers to plant more drought-tolerant crop varieties

or species. Predictions of the El Niño cycle and their practical consequences are thus joined, with strong statistical significance.

El Niño/Southern Oscillation (ENSO) events, which tend to recur every two to nine years, are related oceanic and atmospheric phenomena, characterized by increases in sea surface temperatures of the tropical Pacific Ocean, suppression of upwelling nutrient-rich water along the coast of South America and disruption of the trade winds. The cycle has long been known to be a large component of natural interannual climate variability in the tropics and subtropics, and to a lesser

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FIG. 1 World map showing the areas affected by El Niño (source: N. Nicholls in ref. 4).

extent, in the mid-latitude regions. Global 'teleconnections' (relationships between weather at two or more distant points) linked to ENSO include lower-than-normal precipitation in western Oceania, India, southeastern Africa and northeastern South America, and excessive precipitation in western South America and eastern equatorial Africa² (see Fig. 1). In 1991–93, the last El Niño event (which was of moderate intensity) corresponded with an extremely severe drought in southeastern Africa.

After years of basic research to improve understanding of El Niño mechanisms that has culminated in predictive models³, atmospheric scientists are now collaborating with social scientists to lay the foundations for applied research. In this case, Cane, a leading ENSO modeller, has worked with Buckland, an agricultural economist of the Southern Africa Development Community's Food Security Technical and Administrative Unit, to study the predictability of climate impacts on maize production. The potential for warning farmers in advance of drought and allowing them to prepare for it — not only in Zimbabwe, but in other regions of the world — brings science, literally, right down to ground level.

Such strong climate perturbations have also long been known to have considerable effects on human activities. Indeed, it was the collapse of the anchovy fisheries off the western shore of South America that first brought the El Niño cycle to widespread public awareness in 1972–73. ENSO events, particularly the extreme weather patterns of 1982–83, have been associated with damage to coastal resources, agriculture, transportation, housing and human life in five continents.

Crop productivity (both the quantity and quality of the output) in any location depends on a complex combination of climate, biophysical factors and management. The present study considers only one local climate variable, total annual precipitation. Although this is clearly correlated with crop yield, variations in the frequency, intensity and duration of rainfall all affect a standing crop differently. Deeper understanding of ENSO-driven effects on crop growth, and solving the riddle of why ENSO is more highly correlated with crop yield than with precipitation in Zimbabwe, will depend on

the inclusion of a wider range of variables and timescales. How ENSO affects agriculture also depends on how the biophysical field-level effects (including those on pests) lead to socioeconomic changes. Decisions made by farmers in the face of climate variability ultimately involve the success or failure of individual farms and, by extension, of entire regions, even of national economies highly dependent on agricultural products.

So far, work on ENSO-related impacts,

institutions, including the Inter-American Institute for Global Change Research and the US National Ocean and Atmospheric Agency, are fostering nascent programmes to aid the effective use of ENSO forecasts.

Economists have an important role to play in determining the economic value of climate forecasts. The expected 'value' represents the sum of the net gains to individual countries and groups of an optimal response to the forecasts, as compared with the alternative of 'business-as-usual'. These gains must be weighted by the *ex ante* probabilities of various possible weather and climate conditions. The value of knowledge of the climate system includes not only better agricultural performance, but other benefits such as avoidance of flood damage and improved nutrition and health.

As ENSO climate forecasting grows out of the research mode into operational mode, awareness of and sensitivity to the costs of prediction errors also grows. Although simple correlations between El Niño events and maize yields in Zimbabwe are suggestive, more sophisticated testing is needed. Honesty about the limitations of any forecast is essential, especially when human livelihood is at stake.

What of the immediate future? Zebiak and Cane's current ENSO forecast based on their model⁶ indicates that equatorial Pacific sea surface temperatures will be slightly above normal during November to March (the next growing season in Zimbabwe), but enough to be considered a warm event. This is roughly the consensus of other long-range forecasts, which range from slightly below to somewhat above the mean. The implication is that maize yields in Zimbabwe will be normal or slightly below normal next year. A note of caution: over the past few months the forecasts have tended towards warmer temperatures. Zebiak and Cane advise waiting for September, when the forecast is generally more reliable, to plan agricultural management strategies. □

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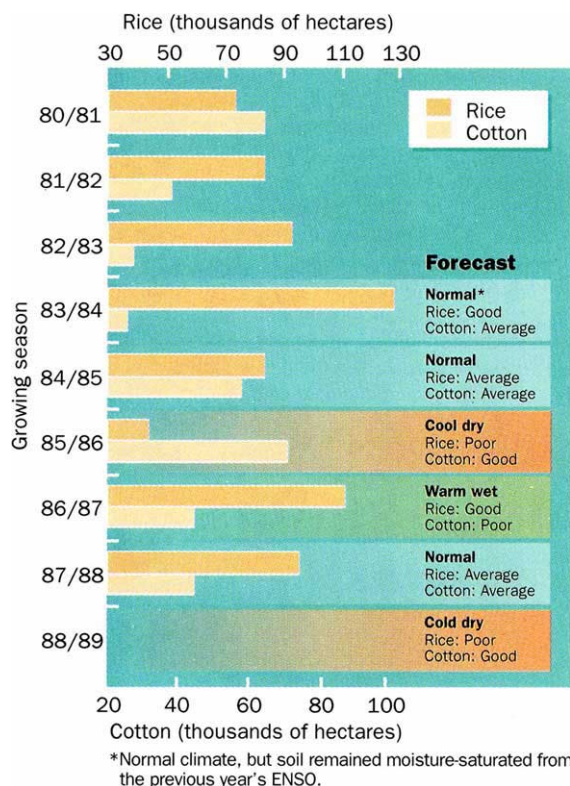


FIG. 2 Agricultural area sown to rice and cotton in the northern coastal region of Peru. ENSO forecasts began before the 1983–84 growing season (source: ref. 5).

including this study by Cane *et al.*, has been done primarily on a country-by-country basis⁴. Although such studies are rich in regional detail, their weakness is that they lack a common context; results can easily become anecdotal. A firmer framework is required, so that knowledge gained in one location (for example, what works and what does not) can be compared and applied in others. The climatological, agronomic and economic datasets and models already available should be marshalled to address the ENSO phenomenon, enabling valuable case studies to be embedded in a larger context.

Predictive activities are already under way in some countries. In Peru, ENSO forecasts are incorporated into national planning for the agricultural sector, and areas planted with rice and cotton (cotton being the more drought-tolerant crop) are adjusted accordingly, as Fig. 2 shows⁵. Governmental and intergovernmental

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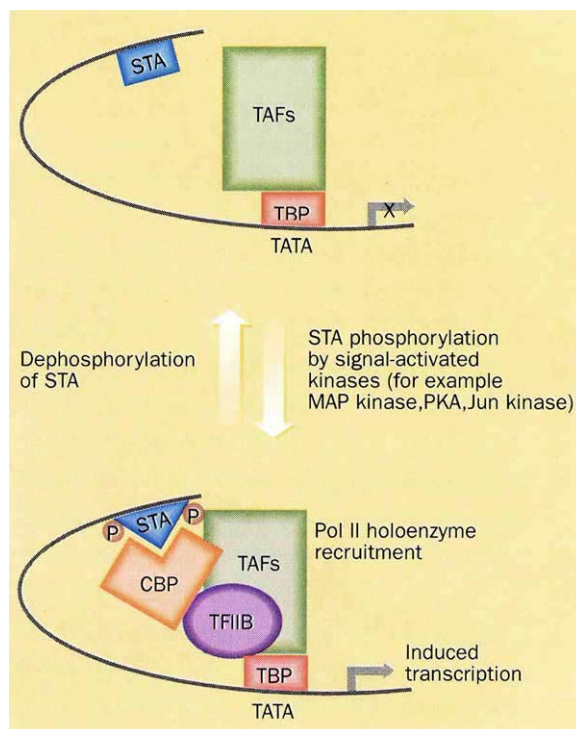
CREB takes CBP to tango

Alfred Nordheim

EUKARYOTIC cell proliferation and differentiation are largely stimulated by extracellular molecules, either free or in the form of cell-surface proteins on other cells, that interact with receptors on the cell surface. Ligand-receptor interaction sets in motion intracellular signalling cascades that cause the rapid transcriptional induction of selected genes. In the final steps of these cascades, specific transcription activators are phosphorylated and bind to sites in the control regions of the required genes to stimulate their transcription. This binding of the phosphorylated activator must then be communicated to the basal transcription machinery sitting some distance away at the start-site of transcription. Two papers on pages 223 and 226 of this issue^{1,2} now identify the CBP polypeptide as a transcriptional coactivator which provides this crucial link between transcription activators stimulated by signalling cascades and initiation of transcription.

Signals that increase intracellular concentrations of cyclic AMP lead to the activation of genes that contain *cis*-regulatory elements termed CREs (cAMP-response elements). Increased cAMP levels cause stimulation and nuclear translocation of protein kinase A (PKA), which subsequently activates the transcription factor CREB (CRE-binding protein) by phosphorylating it at a single residue, serine 133 (ref. 3). This phosphorylation, while it affects neither the intracellular distribution of CREB nor its specific affinity for strong CREs, nevertheless leads to efficient transcriptional activation of CRE-driven genes (for a review, see ref. 4). The molecular mechanisms by which phosphorylation of CREB causes promoter activation have long remained elusive. However, the recent identification of the adaptor molecule CBP (CREB-binding protein)⁵, and now the demonstration of its specific interaction with CREB upon phosphorylation of the serine at position 133 of the latter^{1,2}, provide an important general clue as to how signal-targeted transcriptional activators (hereafter termed STAs) and the basal transcription machinery might connect. Equilibrium binding parameters of the phosphoCREB-CBP interaction, as determined by measurements of fluorescence anisotropy and 'far-western' studies, strongly support the

previous notion⁵ of a phosphorylation-induced increase in the affinity of CREB for CBP^{1,2}. This suggests that phosphoCREB, but not underphosphorylated CREB, functions to recruit CBP, thereby enabling CBP to bridge the distantly positioned CRE-CREB complex and the



Proposed mechanism of transcriptional coactivator function in mediating gene induction by signal-targeted activators. On ligand stimulation, signal-targeted transcriptional activators (STAs), interacting with promoter-distal *cis*-elements, become phosphorylated by signal-activated kinases. Phospho-STA is subsequently able to recruit the coactivator CBP, which itself interacts with TFIIB (and possibly TBP) and further recruits the polymerase II holoenzyme. Dephosphorylation of STAs is postulated here to reverse gene induction and contribute to promoter switch-off.

basal transcription machinery. Indeed, in accordance with this proposed bridging function, CBP is now shown to act as a transcriptional coactivator, augmenting expression of CRE-directed genes^{1,2}.

How then does CBP contact the transcriptional apparatus upon recruitment by phosphoCREB? Kwok *et al.*¹ provide evidence that the activation domain of CBP interacts with the basal transcription factor TFIIB. As TFIIB interacts with the TATA-box-binding protein TBP, and also functions in the recruitment to the promoter of polymerase II holoenzyme⁶, a continuous chain of physical contacts is now established linking the stimulus-activated phosphoCREB, bound distal to the promoter, with the polymerase II complex that initiates transcription.

Participation of TBP itself, and possibly some TAFs (TBP-associated factors), seems probable because of the functional characteristics of TFIIB.

In addition to cAMP-directed signalling, many extracellular ligands, including mitogenic growth factors (for example, EGF and PDGF) and differentiation factors (for example, NGF), activate a host of different intracellular signalling cascades. Much has been learnt in recent months regarding the signalling mechanisms involved⁷. Participating downstream protein kinases include the MAP kinases and Jun kinases, which phosphorylate and activate — among others — the transcription factors Elk-1 and c-Jun respectively^{2,7,8}. Is the CBP coactivator also involved in mediating the effects of these STAs, which function through binding to the *cis*-regulatory sequences SRE (serum-response element) and TRE (TPA-response element) respectively? Indeed, Arias *et al.*² provide evidence that this is the case, showing that signal-dependent phosphorylation of c-Jun at Ser 63 and Ser 73 promotes association with CBP. This, in turn, augments c-Jun-directed transcription. If induced phosphorylation of STAs provides the basis for gene induction by catalysing STA-CBP interactions, then controlled dephosphorylation of STAs and the associated reversal of the STA-CBP interaction offers an attractively simple mechanism for governing the switch-off of signal-activated genes^{9,10}.

CBP is a large protein which has a relative molecular mass of 250,000 and contains a so-called bromodomain^{2,11}, a conserved structural unit thought to be important for protein-protein interactions which is found in *Drosophila* and yeast coactivator proteins involved in signal-dependent, but not basal, transcription. A bromodomain has also been found in another large transcriptional coactivator protein, the recently cloned adenovirus E1A-associated human p300 protein¹²; indeed, even closer homology between CBP and p300 has just been reported¹³. p300 derivatives lacking an intact E1A-binding site can bypass the repressive effect of E1A on SV40 enhancer function and thereby restore enhancer activity¹². This raises the intriguing possibility that coactivators of signal-activated transcription factors might be targeted and thereby deregulated by transforming proteins from DNA tumour viruses. This may represent an important mechanism by which these viral transforming proteins

affect growth and differentiation of infected cells.

The identification of the coactivator CBP as a missing link between the distally positioned, signal-activated transcription factors and the basal transcription machinery offers significant resolution of the molecular mechanisms by which extracellular signals determine gene activity. Phosphorylation-directed protein-protein interactions between STAs and the bridging coactivator CBP provide an explanation for the observation that phosphorylation of latent transcription factors is intimately associated with their activation. Furthermore, it adds an important detail to Ptashne and Gann's¹⁴ discussion of the mechanisms by which enhancers can stimulate promoters from a distance. DNA-bound enhancer proteins seem to connect with the basal transcription machinery indirectly, that is by recruiting

some coactivator which itself interacts with general transcription factors like TBP, as well as TFIIB and the polymerase II holoenzyme. □

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GENETIC IMPRINTING

Taking stock in Stockholm

Rory Howlett

DURING parental gametogenesis, alleles of specific genes can be 'marked' so that their expression depends on whether they have been transmitted through the male or female germ line. A recent meeting in Stockholm* drew together a diverse group of researchers interested in the role of such 'genetic imprinting'.

Imprinted genes function in early development (D. Soltor, Max Planck Inst. Immunology, Freiburg) and are implicated in human genetic disease and cancer. Prader-Willi syndrome (PWS) and Angelman syndrome (AS), clinically distinct disorders resulting in mental retardation, result from disrupted patterns of imprinting. Deletions of chromosome 15q11-q13 are common in both syndromes, but are inherited either paternally (PWS) or maternally (AS). Point mutations in a single gene could be responsible for some cases of AS, and multiple imprinted genes implicated in the aetiology of PWS (R. Nicholls, Case Western Reserve Univ., Cleveland).

An 'imprinting centre' on chromosome 15 possibly resets normal patterns of imprinting in the PWS/AS region: mutations in this centre could result in PWS or AS, depending on whether they first arose in the grandmother's or grandfather's germ line (B. Horsthemke, Inst. Human Genetics, Essen). The centre may reside close to the gene for the small nuclear ribonucleoprotein polypeptide N, a candidate PWS gene (U. Francke, Stanford).

DNA methylation correlates with imprinting in Beckwith-Wiedemann syn-

drome (BWS; M. Mannens, Univ. Amsterdam; R. Weksberg, Univ. Toronto) and Wilms' tumour (WT; A. Feinberg, Johns Hopkins Univ.; A. Reeve, Univ. Otago). Mitotic crossover leading to partial isodisomy may disrupt imprinting patterns in several human cancers (G. Cote, Sudbury General Hospital, Ontario). Relaxation of imprinting on the gene for insulin-like growth factor 2 (IGF2) occurs in BWS and WT, and *H19*, another imprinted gene, is a putative tumour-suppressor gene (B. Tycko, Columbia Univ.).

Despite the plasticity of imprinted patterns (R. Ohlsson, Univ. Uppsala), little is known about genetic variation in imprinting. In mice, the T-associated maternal effect (*Tme*) locus is paternally imprinted and maps close to the insulin-like growth factor 2 receptor (*Igf2r*) locus. The viability of *Tme* maternally derived deletions is controlled by modifier genes that may be involved in *Tme/Igf2r* imprinting (J. Forejt, Princeton).

DNA methylation is important for silencing imprinted genes, and could be the primary event in establishing the imprinted pattern, perhaps affecting the composition of chromatin. Alternatively, methylation could be secondary to epigenetically inherited chromatin modifications (A. Wolffe, NICHD, Bethesda). Expression of genes can depend on their position in relation to different types of chromatin. For example, factors that suppress mutations in the *white* locus of *Drosophila* may influence the extent of repressive heterochromatin near *white*

transgenes (T. James, Wesleyan Univ., Connecticut).

In mammals, an X-inactivation centre helps silence one of the two X chromosomes inherited by a female zygote (M. Lyon, MRC Radiobiology Unit, Harwell): chromosome segments next to an 'unblocked' centre are inactivated. A candidate for the inactivation centre is *Xist*, a gene expressed only from the otherwise inactive X chromosome. The expression of the gene is thought not to be sufficient for inactivation, but does stabilize the effect. *Xist* has no substantial open reading frame, and its transcribed RNA is retained in the nucleus, perhaps interacting with proteins and/or nucleic acids.

Both germ-line modifications and post-zygotic processes are involved in imprinting, and A. Surani (Wellcome Inst., Cambridge) has compared these for *Xist* and the enigmatic *H19*, the RNA of which is found in cytoplasmic particles. In cultured mouse embryos, bi-allelic expression of *H19* becomes mono-allelic after postzygotic modifications to the gene. Critical sites in the *H19* promoter are hypermethylated in the paternal allele in the post-zygote but the paternal allele is still expressed in early zygotes, expression falling off only with time. By day 4.5, both copies are unmethylated in the trophectoderm, but by day 6.5 the paternal allele becomes methylated. In the case of *Xist*, Surani finds expression of the paternal copy at the four-day stage. Methylation of the maternal allele is repressed in the early embryo. But the imprints are temporally labile, and the early expression of *Xist* may depend on a maternal factor that becomes depleted early in development.

W. Reik (Babraham Inst., Cambridge) finds methylation upstream of the imprinted *Igf2* gene. The imprinted allele is about twice as methylated as the expressed allele, but Reik thinks it unlikely that specific differences in methylation alone are responsible for the germ-line alone imprint. Transgenes bred into different mouse genetic backgrounds obtain different patterns of methylation (W. R. and N. Allen, Babraham Inst., Cambridge), and genetic crosses suggest the action of imprinted modifier genes.

Imprinting in the *H19* and *Igf2* region has also been scrutinized by S. Tilghman (Princeton). Methylation maintains the imprint on the paternally derived *H19* allele and the gene is relatively heavily methylated in the sperm. Contrary to Surani's results with cultured embryos, however, the retention of some specific methyl groups is enough to repress the paternal allele in natural early embryos. As strong enhancers of *H19* also affect *Igf2*, an attractive model portrays competition between these and other imprinted genes in the region, such as *MASH2* and *insulin 2* for enhancers, providing the basis for coupled regulation.

*Nobel conference on Parental Imprinting: Causes and Consequences, Stockholm, Sweden, 12–14 June 1994.

Mapping the Milky Way

Philipp P. Kronberg

At least two regions of the *Igf2r* gene carry parent-specific methylation patterns (D. Barlow, Inst. Molecular Pathology, Vienna). Sites in the promoter region are methylated on the repressed chromosome, whereas a second region is methylated on the active chromosome, perhaps blocking a transcriptional repressor. Barlow finds no evidence for a highly conserved 'imprinting box' common to other imprinted genes. Instead, she suggests that primary imprinting sites could look like 'foreign' DNA, and that imprinting could originally have evolved as a kind of 'host defence' against the genomes of pathogens. Alternatively, imprinting could have evolved in response to intragenomic conflicts resulting from the expression of certain genes having different fitness consequences depending on whether they come in eggs or sperm (D. Haig, Harvard).

DNA methyltransferase is presumably only one of the many enzymes present during the replication of DNA, some of which may mediate the clonal transmission of chromatin structure (T. Bestor, Harvard). Essential sequences target the protein to replication foci, and a fluorescent tag is being used to follow the behaviour of the enzyme in live cells. Different enzymes probably lay down and maintain the methylation pattern of imprinted genes. By manipulating genomic methylation in embryos mutant for DNA methyltransferase (R. Jaenisch, Whitehead Inst.), abnormally low methylation levels have been found that kill embryos but not their stem cells, suggesting the presence of at least two activities — a *de novo* methyltransferase that establishes the imprint and a hemimethylase required for its maintenance.

In mice, at least ten regions on six chromosomes are subject to imprinting (B. Cattaneach, MRC Radiobiology Unit, Harwell). Evidence for 'domains of imprinting' also come from the experiments of H. Cedar (Hebrew Univ., Jerusalem), who has identified timing zones in the ordered replication of chromosomes. In the case of imprinted genes such as *Igf2*, the maternally and paternally derived alleles seem to replicate asynchronously. Moreover, studies of the region containing the PWS and AS loci, for example, suggest the coordinated replication of large imprinting domains.

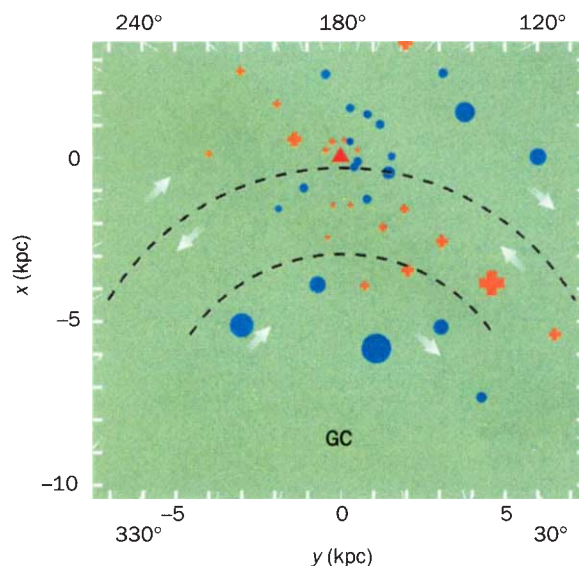
T. Mukai (National Cardiovascular Centre Research Inst., Osaka) has developed a methylation-sensitive genome scanning method to identify new imprinted genes, from which the number of imprinted genes in the average mammalian genome is estimated to be about 200. But this figure is not for imprinting just yet. □

Rory Howlett is an associate editor at Nature.

A RECENT paper by Rand and Lyne¹ describes a noteworthy new attempt to define the large-scale structure in the plane of the Milky Way using radio waves from pulsars within our Galaxy. The first inkling that the Milky Way is permeated by a large-scale magnetic field came from Faraday rotation measures (RM) of the polarized radiation of extragalactic radio galaxies and quasars. As polarized radio waves from these sources enter the Milky Way, a 'local' RM is imposed. Early data of this sort were first used for this purpose in 1968 by Davies², who concluded that our 'local' neighbourhood in the Galaxy has a surprisingly ordered and widespread magnetic field, which is oriented at approximately 90° to the direction of the Galactic Centre. Better radio data collected in the late 1970s by Simard-Normandin and myself³ confirmed this finding, and revealed that the ordered nature of our Galaxy's field extends throughout the entire Milky Way plane, and furthermore that there is at least one large-scale zone in which the prevailing field direction reverses its sign (in the direction of the Sagittarius constellation).

RM measurements of pulsars (located within the Galaxy) are another, more advantageous, way of probing the large-scale galactic magnetic field. This is because pulsars lie at differing distances within the Milky Way plane, and because they provide an additional measurement, that of the dispersion of radio waves by electrons, which also allows distance estimates for the pulsars — and hence a determination of their three-dimensional distribution within the Milky Way. Furthermore, as RM is proportional to $\int n_e B_{\parallel} dl$ (where n_e is the electron density and $B_{\parallel}$ the magnetic field component parallel to the line of sight), and the dispersion measure (DM) is proportional to $\int n_e dl$, the quotient RM/DM for the same pathlength, l_p , which is the distance to a pulsar, directly gives a weighted strength of the line-of-sight component of the galactic magnetic field along the path to the pulsar in question. This contrasts with the situation for extragalactic-source RMs, which probe the entire galactic sightline in the direction

of the extragalactic source in question, and for which we need independent information on the column density ($\int n_e dl$) of ionized gas along these galactic sightlines to estimate the magnetic field strength. Even with such estimates, extragalactic sources, unlike pulsars, do not offer the opportunity of probing the magnetic field along sightlines of varying length within the galactic plane.



Values of Faraday rotation measure (RM) for radio waves from galactic pulsars averaged in 20 bins of longitude and in distance bins 0–1 kiloparsecs (kpc), 1–2 kpc, 2–3 kpc, 3–5 kpc, 5–7 kpc and 7–10 kpc. Positive RMs are depicted as orange crosses, with increasing cross size representing increasing magnitude (in a range 25 to 300 rad m^{-2}). Negative RMs are depicted by blue circles, with increasing circle size representing decreasing magnitude (in a range –25 to –300 rad m^{-2}). Overlaid dotted line is a sketch of the best-fitting ring model of the galactic magnetic field, showing the two reversals. GC, Galactic Centre; triangle, our location within the Galaxy. (Figure adapted from ref. 1.)

The problem up to now has been that the pulsar RMs have been more difficult to measure than DMs and extragalactic-source RMs, and only now are enough available to probe the Galaxy's large-scale field structure independently of the more numerous extragalactic-source RM data. The Rand and Lyne paper¹, with its 27 new pulsar RMs, provides just enough RM/DM ratios to begin to define the large-scale field geometry within the galactic 'forest', which we are condemned to observe only from within. Their paper nicely illustrates the types of analyses that will be possible in future, when the galactic plane area is more densely covered with pulsar RM and DM measurements. As more pulsar RMs become available, they will provide a powerful probe of the magnetic field structure and strength over the plane of the Milky Way.

The figure illustrates the result, which, at this point, confirms the earlier evidence³ for a large-scale field reversal in the vicinity of the Sagittarius Arm. However, their 'map' of our Galaxy's magnetic field now provides much better detail in the 'plan view' of the galactic field geometry in the first quadrant of the galactic plane.

The confirmation of a field reversal provides an important constraint on dynamo theories, which attempt to describe how the large-scale galactic field evolved over cosmic time. Different versions of the dynamo theory produce either an axisymmetric field distribution (in which there are no large-scale systematic reversals), or bisymmetric galactic fields, which have large-scale reversals, or more complex global field patterns. Most of the 20-odd spiral galaxies in the 'nearby' Universe whose large-scale magnetic fields have been investigated seem to have axisymmetric fields. Rand and Lyne's results thus indicate that the Milky Way is among the 'bisymmetric field minority' among nearby galaxies.

However, the latest observational indications are that intermediate cases may well also occur, in which the magnetic field direction reverses only over limited regions, not globally within a galaxy's disk. Some theoretical calculations suggest that such subglobal reversals may be 'transient' features over a galaxy's 10^{10} year lifetime. If the latter idea is borne out by future RM observations and theory, then the current global field distribution in galaxies may not, after all, contain a memory of the primordial field orientation at the time the galaxy formed. This question is an example of several reasons why it is interesting to define the strength and geometry of the large-scale magnetic field structure in galaxies. Pulsars will enable us to do that in increasingly fine detail for our own Milky Way.

The measurement of actual magnetic field strengths along various pathlengths through the Galaxy is also of great interest — in particular in deciding the currently debated question of how, and at what epoch in the history of the Universe, galactic magnetic fields were amplified up to their current, relatively strong levels. Through a variety of measurement techniques, including pulsar RM/DM ratios, interstellar magnetic field strengths are known to be of the order of 2–5 μG at the Sun's radius (~ 9 kiloparsecs from the Galactic Centre). These results reveal that the magnetic energy density in the interstellar medium is comparable to that of the cosmic (extragalactic) microwave background density, the local cosmic-ray

energy density, and also (coincidentally?) that of the general starlight in the Milky Way. However, the causes and effects that may have determined these similarities are very much unknown at present. Future pulsar RM/DM ratios towards the outer Galaxy will solve the very interest-

ing question of if, and by how much, the galactic magnetic field strength decreases toward the periphery of our Galaxy. $\square$

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TUMOUR-SUPPRESSOR GENES

Open questions on p16

Laura Bonetta

AT the beginning of last year, the very existence of the cyclin-dependent kinase (CDK) inhibitors was unsuspected. By the end of it, it had become clear that these proteins are critical regulators of the cell cycle and act as 'brakes' at various points. Could removing the brakes throw the cell into unregulated proliferation? The discovery that the CDK inhibitor p16 is homozygously deleted in a striking proportion of human tumour cell lines^{1,2} suggested that it could, and raised the possibility that p16 might be a major contributor to cancer development. It now seems that things are not so simple. On page 183 in the Scientific Correspondence section of this issue³, Spruck *et al.* show that although p16 deletions are indeed frequent in tumour cell lines, they are much less common in primary tumours.

The gene for p16 lies on chromosome 9p21. This region is frequently rearranged or deleted in primary human tumours, and often shows loss of heterozygosity, strongly suggesting that it includes a tumour-suppressor gene analogous to the retinoblastoma or p53 genes. When the status of the gene encoding p16 (also known as *MTS1*, for multiple tumour suppressor 1) was examined, it was found to be deleted in about half of the cell lines analysed^{1,2}. Several melanoma cell lines were also found to have missense and nonsense mutations within the gene², indicating that p16 might be the critical tumour-suppressor gene in 9p21.

But these initial studies looked at cell lines, which are notoriously prone to genetic rearrangements as a result of culture conditions. Spruck *et al.* have now examined primary cells from bladder tumours for p16 alterations: only 6 out of 31 bladder tumours had homozygous deletions in the p16 gene, and only one had a point mutation, indicating that p16 defects are three times less common than in the cell lines.

These results raise two issues. First, despite the fact that only 19 per cent of primary bladder cancers have homozygous deletions in p16, 80 per cent of such tumours show loss of heterozygosity at 9p21. So there may be other tumour-suppressor genes in this region, which would also be affected by these deletions.

Second, the discrepancy between the mutation frequencies seen in primary tumours and in cell lines demands explanation. Although several hypotheses have been put forward, other work published this month shows that the frequency of mutations seems to vary with the tumour type. Cairns *et al.*⁴ looked for p16 mutations in various primary tumours in which the 9p21 region had lost heterozygosity but the p16 gene was not deleted. Out of 75 tumours examined, none of which were melanomas, only two carried somatic mutations in p16. By contrast, Mori *et al.*⁵ looked specifically at oesophageal squamous cell carcinomas and found somatic mutations of p16 in 14 out of 27 tumours.

But the discrepancy may also reflect the ease with which cell lines can be derived from the tumours in question (just as the *N-myc* gene is amplified in every neuroblastoma cell line examined so far). Spruck *et al.* show that in three cell lines for which normal and tumour DNAs were available, the p16 gene was already altered in the corresponding tumours. Of the 13 cell lines examined, 12 had alterations in either the p53 or the p16 gene (the remaining cell line had a mutation in *Ha-ras*), implying that lesions in either p53 or p16 confer a long-term growth advantage in culture.

What is the bottom line? p16 is obviously involved in controlling cell growth, and some primary tumours indeed have nonsense mutations and deletions within the p16 gene. Its contribution to tumorigenesis, however, may not be as significant or as general as initially anticipated. The final verdict awaits a comprehensive analysis of primary tumours with loss of heterozygosity in 9p21 from which cell lines have been derived, comparing point mutation and deletion frequencies. The answer may well be that p16 is important in some types of tumours but not others. $\square$

Laura Bonetta is an assistant editor at Nature.

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Imbalance in the budget

Fortunat Joos

A TOP priority in global change research is to improve our understanding of the global carbon cycle. Fossil fuel emissions and other anthropogenic activities have increased atmospheric carbon dioxide by almost 30 per cent in the past 200 years. For the past decade, on average about half of the estimated 6.6 gigatonnes of carbon released per year ($1 \text{ Gt} = 10^{15} \text{ g}$) has remained in the atmosphere. Less certain is how the remaining carbon is distributed between the other reservoirs. According to model estimates, about 2 Gt enters the ocean yearly, while the rest (about 1.2 Gt) is attributed to an unidentified terrestrial sink. Understanding just how the mechanisms for carbon uptake operate is crucial.

On page 201 of this issue¹, and in work presented at a conference last year², Hesshaimer, Heimann and Levin challenge our quantitative understanding of the global carbon cycle. They find that, using present estimates, they cannot balance the global budget of artificially produced radiocarbon. To correct this, they suggest that ocean uptake estimates based on observations made during the GEOSECS campaign³ must be revised downwards, and that a similar decrease should be fed into model-based estimates for ocean uptake of anthropogenic CO_2 .

Inadvertently, the atomic bomb tests of the late 1950s and early 1960s have yielded valuable geophysical information. The bomb tests added substantial amounts of radiocarbon to the natural background ($^{14}\text{C}/^{12}\text{C} \approx 10^{-12}$), leading to maximum tropospheric concentrations around 1964. Once test-ban treaties were set in place, stratospheric and tropospheric levels decreased as bomb radiocarbon became absorbed into the oceans and biosphere. Tracking the injection of bomb radiocarbon into the stratosphere and its subsequent spread into other carbon reservoirs allows one not only to study the global carbon cycle, but also to gain insight into the dynamics of the upper ocean and into tracer exchange between stratosphere and troposphere.

The ocean's radiocarbon distribution was mapped systematically, along with many other tracers, during the global GEOSECS campaign (1972–78). Today, these and many recent data serve as a standard to validate and calibrate ocean models, and estimates of ocean CO_2 uptake rely heavily on measurements of the penetration of bomb radiocarbon^{3,4}.

In their new study¹, Hesshaimer *et al.* investigate the global bomb-radiocarbon budget and point out a problem that, surprisingly, has been long overlooked. The problem involves two distinct time periods. First, in the late 1950s and early

1960s, bomb testing was at its highest: most of the bomb radiocarbon was still in the atmosphere. By combining atmospheric observations and bomb test statistics, they calculate a yield factor that allows them to convert estimates of explosive power into radiocarbon production. Second and more important, after the test ban atmospheric explosions were relatively rare, so the global radiocarbon budget should have remained roughly constant, an additional constraint that must be satisfied by carbon cycle models. Hesshaimer *et al.* find that an extra radiocarbon source equivalent to about 80% of the biospheric uptake or 25% of the oceanic uptake needs to be introduced to restore balance in their model.

Of the four important carbon reservoirs, global radiocarbon observations exist for three: the troposphere, the stratosphere and the ocean. The tropospheric concentration has been monitored since the onset of the bomb tests. Stratospheric data exist until 1969 and for 1989. For the

global ocean, observations exist only for the mid-1970s. Hesshaimer *et al.* derived the oceanic uptake history by using Siegenthaler and Oeschger's box-diffusion model⁵ as calibrated to match the observed bomb-radiocarbon distribution at the time of GEOSECS. To estimate the uptake of the remaining biospheric reservoir, a three-box model accounting for soil, short-lived and long-lived vegetation was used. The global biospheric uptake of bomb radiocarbon cannot be easily assessed by observation, but as it is of the same order as the imbalance, there seems to be no reasonable way to balance the budget purely by changing the model's biosphere structure.

How then can the imbalance in the bomb-radiocarbon budget be explained? Hesshaimer *et al.* provide substantial evidence suggesting that the accepted ocean inventory should be lowered by 25%. This, however, conflicts with a preliminary 15% upward revision of the inventory based on a more refined analysis of the GEOSECS observations^{3,6}. With that, balancing the budget becomes even harder.

Broecker and Peng⁶ have confirmed that in their model there is an imbalance in

Reservoir	Change in inventory mid-1965 to mid-1989 (10^{26} atoms)	Comments
Biosphere	$+75 \pm 30$	Average of 4 models in refs 1, 3, 5, 10. Model results: 60, 45, 89 and 99 units
Ocean	$+310 \pm 60$	Average of 3 models in refs 1, 6, 10. Error based on uncertainty in inventory estimates ^{3,6} . Model results: 299, 337 and 287 units
Troposphere	-150 ± 15	Derived from observations; assumed known within 10%
Stratosphere	-100 ± 30	Derived from observations ⁷ ; error of roughly 30% assumed because original estimate for 1965 has since been revised downwards ^{1, 11} by 17%
Changes in all reservoirs		$+135 \pm 75$
Sources and sinks	Cumulative production 1965 to 1989 (10^{26} atoms)	Comments
Nuclear industry	3 ± 2	Amount negligible
Radioactive decay	-1.7 ± 0.7	Amount negligible; calculated using global inventory of 6×10^{28} atoms and half-life 5,730 years
Bomb detonation	52 ± 20	See Hesshaimer <i>et al.</i> ¹ for calculation
Total production	53 ± 20	
Imbalance*	82 ± 78	
Data for production by nuclear industry and bomb detonation, and for atmospheric observations, courtesy of V. Hesshaimer. Model results for ocean and biosphere uptake courtesy of V. Hesshaimer and T. H. Peng.		
*Error obtained by quadratic error addition.		

bomb-radiocarbon budget for the post-test-ban period and, like Hesshaimer *et al.*, suspect that the reason lies in the ocean. However, they give a somewhat different explanation. Instead of lowering oceanic uptake estimates for each year, they propose an enhanced uptake for the bomb-test period, and a reduced uptake after 1963. This allows them both to fulfil the constraint of a roughly constant global ^{14}C -inventory for the post-test-ban period, and to match their GEOSECS inventory estimate^{3,6} for the mid-1970s. The initial enhanced oceanic uptake, which by 1963 accumulates to an increase in model inventory of roughly 100%, would probably require a substantially increased gas exchange rate (the uptake is largely limited by air-sea exchange). Increasing the gas exchange is not without its problems, as radiocarbon-based estimates of the global CO_2 gas exchange rate are already substantially higher than estimates based on wind tunnel and open lake experiments.

The uncertainties in all these figures are large. The tables list changes in bomb-radiocarbon inventories from 1965 to 1989, for which the budget imbalance amounts to $(82 \pm 78) \times 10^{26}$ atoms (82×10^{26} represents 10–15% of the global inventory). Most of the budget uncertainty stems from the ocean uptake estimate, because almost half of the bomb radiocarbon entered the ocean during that period. Although error estimates are somewhat arbitrary, the large uncertainty for this imbalance term implies that major revision of our picture of the global carbon cycle would be premature. But the effort to clarify the bomb-radiocarbon budget must be made if we are to refine our understanding of what controls the distribution of anthropogenic CO_2 .

What are the consequences of this imbalance? Hesshaimer *et al.* further suggest that the air-sea gas exchange, the bomb-radiocarbon penetration depth and thus the oceanic CO_2 uptake should also be lowered by 25%. However, bomb-radiocarbon and anthropogenic CO_2 do not behave identically. First of all, the equilibration time between surface water and air is about ten times longer for ^{14}C than it is for anthropogenic CO_2 , so the bomb-radiocarbon inventory strongly depends on gas exchange. In contrast, CO_2 uptake is more limited by mixing between surface and deep ocean waters. The bomb-radiocarbon penetration depth characterizes this downward transport of anthropogenic CO_2 to a large extent⁴, but not perfectly. The difference in the downward mixing of anthropogenic CO_2 and ^{14}C arises from the difference in time-scales: in surface water, CO_2 concentration has grown for the past 200 years with a 30–60 year *e*-folding timescale, whereas bomb radiocarbon increased rapidly in just two decades to reach its maximum in

the early 1970s.

The link between bomb-radiocarbon inventory and CO_2 -uptake estimates needs to be considered carefully. It is probable that there is a correlation between errors in the estimated bomb-radiocarbon inventory and in surface concentration (estimated as the difference between pre- and post-bomb measurements). So a simultaneous downward revision of bomb-radiocarbon surface concentration and inventory estimates does not necessarily require an equally large revision of their ratio, the penetration depth (defined as inventory in atoms m^{-2} divided by surface concentration in atoms m^{-3}). One would first need to quantify the relation between new estimates of ^{14}C inventory and penetration depth, before treating the bomb-radiocarbon penetration depth as a good measure for the ocean's anthropogenic CO_2 uptake. The bottom line is that a revision of CO_2 uptake estimates may be substantially smaller than the 25% suggested for the bomb-radiocarbon inventory.

Bomb radiocarbon, then, retains its usefulness as a benchmark for ocean models of CO_2 uptake, but it cannot be used alone. We need independent confirmation of mixing rates obtained by monitoring the penetration of CFCs and other transient tracers into the ocean. Also important are efforts to measure changes in atmospheric oxygen⁷ and atmospheric and oceanic ^{13}C (ref. 8). These two tracers are linked inextricably to the cycling of carbon, and their monitoring will help to distinguish carbon fluxes between atmosphere and ocean from those between atmosphere and biosphere. Combining all these pieces of the puzzle should produce a more detailed picture of carbon's pathway through the global climate system.

One last note. Uncertainties of the kind discussed above are thoroughly considered in the present scientific assessment of the Intergovernmental Panel on Climate Change⁹. It remains a fact that atmospheric CO_2 will continue to grow if carbon emissions are not lowered. □

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Draining away

MANY people, mostly women, seek to enhance their charms by having their excess fat surgically removed. Unlike dieting or exercise, liposuction is rapid, and can be directed at specific areas of fat. But it can also be expensive, crude and painful, and the results are not always pleasing. Daedalus now has a new idea.

Body fat, he points out, is an ester of glycerol and fatty acids. When the body needs energy, the fat is hydrolysed to glycerol and free fatty acid, which is released into the blood as a biological fuel. This fuelling system can react very rapidly. At any moment there is only about a gram of free fatty acid in the bloodstream, but if used up it can be replaced in a few minutes. So, says Daedalus, instead of removing fat surgically, why not extract it continuously from the blood? A gram of weight could be lost every few minutes. A few hours of the treatment every day could lose a pound of fat per week.

The obvious technology for the job is the dialysis treatment used to purify the blood of people with kidney failure. The patient would merely sit or lie down with a couple of transfusion needles in her. One would extract her blood and pass it through a fatty-acid separator; the other would reinject the defatted blood. No vigorous action or iron self-control would be required.

Compared to surgery, this simple and almost non-invasive process has one serious disadvantage. It gives no control over the region of fat to be lost. Women suffering from the dreaded pear-shaped syndrome, or men burdened with a spare tyre, could not be sure of losing fat from the afflicted region. But Daedalus recalls that the metabolism of fat rises with temperature, possibly because its viscosity drops. This may be why many figure-shaping programmes specifically exercise the muscles in the fatty regions. Sadly, the resulting muscular heat spreads all round the body and has no local effect. But Daedalus's blood-defatting process could easily be refined by warming the areas to be lost and cooling those to be retained. The body could be truly sculpted at last.

To attain the figure of her dreams, the subject would have to remain immobile and plumbed into the system for several hours a day. She would wear heating blankets over the regions to be lost, and water-cooled sheeting over those to be retained. This extended ordeal may seem daunting; but Daedalus points out that many people already stay still for at least four hours a day, watching television. The slight extra distress would probably go unnoticed.

David Jones

p16 gene in uncultured tumours

SIR — Two recent reports have identified a high frequency of homozygous deletions in the gene encoding the p16 protein (*MTS1*), which is an inhibitor of cyclin-dependent kinase-4 (CDK4), in cell lines derived from human tumours of diverse histological types^{1,2}. Additionally, several apparent mutations in the p16 gene were identified in cell lines derived from melanomas¹. These mutations could possibly cause gene inactivation in cells which had not undergone a homozygous deletion for chromosome 9p21, where the gene resides. The p16 gene is an attractive candidate for a tumour-suppressor gene, because loss of its normal function as an inhibitor of CDK4 (ref. 3) would be expected to lead to uncontrolled cell growth.

The use of cell lines facilitates the detection of homozygous deletions of genes. However, only a minority of tumours form permanent cell lines upon explant, and adaptation of cells to culture has unknown genetic consequences. Furthermore, it is not always possible to distinguish disease-causing mutations from naturally occurring polymorphisms without access to the patients' somatic DNA. Because loss of heterozygosity of chromosome 9 is a common genetic alteration in human bladder cancer, we examined a series of 31 uncultured bladder tumours and paired white blood cells (WBCs) as well as 13 bladder cancer cell lines for the occurrence of p16 gene homozygous deletions and mutations. As we have previously examined the same samples for p53 gene mutations, we also compared individual cell lines for the concomitant appearance of genetic damage in both genes. Also, as some of the cell lines were derived by us, we investigated whether p16 gene mutations and deletions were induced or selected for during the establishment of cell cultures.

Homozygous deletions of the p16 gene were detected by quantitative polymerase

chain reaction (PCR) using primers flanking exon 2, and standardized for DNA input with primers specific for the polymorphic GSN dinucleotide repeat marker on chromosome 9q. Homozygous deletions were confirmed by Southern blot analysis using the p16 complementary DNA as a probe³. Exon 2 of the p16 gene, where most mutations have been detected previously, was analysed by direct sequencing.

p16 gene alterations were found to be

TABLE 1 HOMOZYGOUS DELETIONS AND MUTATIONS IN THE p16 GENE IN CULTURED AND UNCULTURED BLADDER TUMOURS

	No. tested	No. with p16 gene deletions	No. with new mutations	% With p16 gene alterations
Cell lines	13	5	2	54%
Tumours	31	6	0	19%

Deletions of the p16 gene were analysed by PCR with primers flanking exon 2. The polymorphic GSN marker located on chromosome 9q was used as a reference. Homozygous deletions were assigned to tumours in which the p16 PCR product was reduced to less than 20% of the GSN control. All deletions were confirmed by Southern blot. p16 gene mutations in exon 2 were detected by direct sequencing.

TABLE 2 p16 AND p53 STATUS IN BLADDER CANCER CELL LINES

Cell line	p16 gene status	p53 gene status	Comments
LD71	Deleted	Wild type	Tumour not available
LD137	Wild type	Mutated	Tumour not available
LD583	Deleted	Wild type	Tumour also deleted for p16 gene
LD600	Mutated (GAC→AAC at nucleotide 214)	Mutated	Tumour also contained identical p16 and p53 gene mutations. WBCs did not contain p16 gene mutation
LD605	Deleted	Wild type	Tumour also deleted for p16 gene
RT4	Deleted	Wild type	
J82	Wild type	Mutated	
HT1376	Wild type	Mutated	
TCC SUP	Wild type	Mutated	
HT9	Wild type	Mutated	
SCaBER	Mutated (2-bp deletion between nucleotides 224 and 227)	Wild type	
UMUC-3	Deleted	Mutated	
T24	Wild type	Wild type	Cell line has Ha-ras mutation

The LD series of cell lines was established from tumours cultured in our laboratory and lines were checked for possible cross-contamination by comparing the WBCs and original tumour with the corresponding derived line using highly polymorphic DNA markers. The other cell lines were obtained from the American Type Culture Collection, Rockville, MD. p53 gene mutations in exons 5–8 were detected and sequenced as described previously⁸ except for the TCC SUP line where the mutation was detected as a truncated protein after immunoprecipitation.

three times more common in cell lines than in uncultured tumours (Table 1). Statistical analysis confirmed a significant difference in the frequency of appearance of alterations between the two groups ($P=0.033$, two-sided). Two of the 31 bladder tumour patients examined had G→A transitions at nucleotide 436 (Ala→Thr) and one had a G→T transversion at position 373 (Ala→Ser) in their WBCs, and these changes were not scored as mutations. In addition, in two of the three patients with these p16 gene alterations in the WBCs, the p16 gene had been homozygously deleted in the tumours. Thus, the presence of the altered gene did not necessarily give the tumour a sustained growth advantage. The nucleotide 436 transition has been previously reported as a mutation in a melanoma cell line¹ but now seems more likely to be a polymorphism.

The results obtained with the cell lines are shown in Table 2. First, it seems remarkable that 12 of 13 cell lines examined contained an alteration in the p16 gene or the p53 tumour-suppressor gene. It is possible that either one of these lesions confers a long-term growth advantage in culture. Neither alteration was present in the T24 cell line, which is known to contain a Harvey-ras mutation⁴, and only two lines were found to contain defects in both genes. A second important point is that the p16 gene deletions in lines LD583 and LD605, in which WBC and tumour DNAs were available for analysis, were already present in the corresponding tumours. The p16 gene mutation detected in line LD600 was also found in the corresponding tumour but not in the matching WBCs, providing the only direct evidence of this gene's potential involvement in tumorigenesis. This information, coupled with the higher rate of occurrence of p16 gene alterations in cell lines, strongly suggests that establishment of cell lines is more easily achieved with those tumours containing p16 gene alterations.

Work from several laboratories has pointed to the importance of chromosome 9 in human bladder carcinogenesis^{5,6}. It has been proposed that at least two tumour suppressor genes relevant to bladder cancer reside on this chromosome⁷, and it will be of interest to determine whether other candidate genes are present in the 9p21 deleted region. It is important to remember, however, that although p16

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gene deletions were less frequent in uncultured than in cultured tumours, they were still present in 19 per cent of tumours examined. It will also be of interest to determine the two pathways by which p16 and p53 gene defects facilitate growth *in vitro* in bladder and other tumour types.

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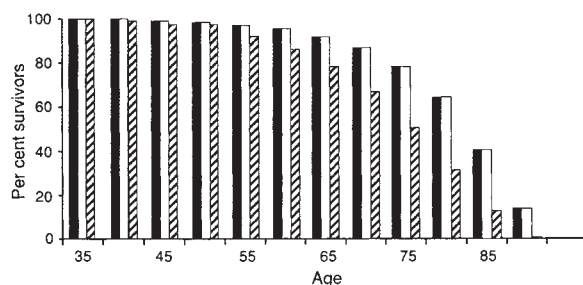
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Tobacco-related mortality

SIR — Despite widespread knowledge of the health risks of cigarette smoking, many smokers cannot quit because they are addicted to nicotine. B. R. has proposed earlier¹ that inveterate smokers switch to oral smokeless tobacco, consumed as chewing tobacco or moist snuff,



Survival rates for 35-year-old individuals: smokers (hatched bars), non-users of tobacco (black bars) and smokeless-tobacco users (open bars).

which also satisfies a smoker's nicotine addiction. This strategy has not been pursued as a public-health measure because of concern that smokeless tobacco might cause some people to start or to recommence smoking, and because it poses a health hazard. But the only consequential hazard from smokeless tobacco use known so far is oral cavity cancer². In India, use of smokeless tobacco (although usually combined with betel leaf, areca nut, and/or slaked lime) is a major risk factor for oral cancer. Yet in the United States, its use has only a small risk³. Even prolonged use of the newer smokeless tobacco products may have a low absolute risk, which would not comprise a meaningful objection to smokeless tobacco substitution for the inveterate smoker.

We estimated the life expectancy of 35-year-old white males with three patterns of tobacco use: non-users, cigarette smokers and smokeless-tobacco users.

For non-users and smokers, we used the mortality rates from the American Cancer Society's second cancer prevention study of smoking and mortality among more than one million Americans⁴.

We employed standard methods to determine the attributable risk for oral cancer among smokeless-tobacco users⁵. A relative risk of 8 (oral cancer risk among users relative to that among non-users) was used to estimate the excess oral cancer mortality in this group. We applied the excess risk equally at all ages after 35 years, even though oral cancer generally occurs in users over age 70 (ref. 6).

The results indicate that the average remaining life expectancy of a 35-year-old smokeless-tobacco user is 45.92 years, only 0.04 year less than that of a non-user (see figure). This 15-day reduction in life expectancy is in sharp contrast to the 7.8 years lost by smokers. Thus, both the 35-year-old non-user of tobacco and the smokeless-tobacco user will live on average to be 80.9 years of age compared with 73.1 years for the smoker. Only 67 per cent of smokers will be alive at age 70, compared with more than 87 per cent of smokeless-tobacco users and non-users of tobacco. Although the effect of smokeless-tobacco on life expectancy may

at first seem surprising, the absolute risk of developing oral cancer from its use is small, the disease is not uniformly fatal, and this type of tobacco use is not associated with the other causes of smoking-related deaths.

Experience of the past 30 years in the United Kingdom and the United States shows that, despite substantial reductions in smoking uptake and continuation rates, many people remain addicted to nicotine, often

with fatal consequences. We suggest that abstinence is not the only approach to reducing tobacco-related mortality: for smokers addicted to nicotine who would not otherwise stop, a permanent switch to smokeless tobacco could be an acceptable alternative to quitting.

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Electrostatic sense in rattlesnakes

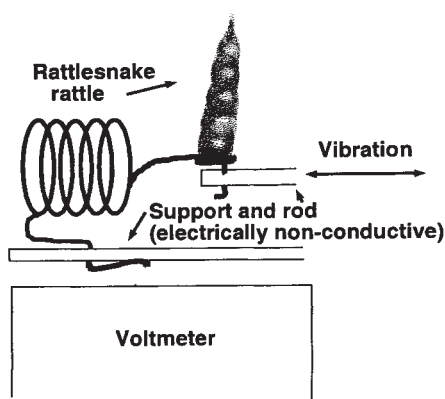
SIR — An isolated rattlesnake rattle produces a positive electrostatic charge of 75 V when mechanically vibrated. Friction of snake skin on materials common in the environment also produces positive charges as high as 1,000 V. These facts are a necessary condition for the hypothesis that snakes sense the presence of cover and other environmental features by electrostatic effects involved in tongue scanning. There are two other considerations.

First, in common with all other dry-skinned land animals, snakes acquire electrostatic charges by friction during their sliding movements and are typically observed to have positive charge levels of between 100 and 1,000 V. Possibly because of electrostatic discharge hazards plus the tendency of charged surfaces to attract dust, all dry-skinned land animals other than snakes and snake-like lizards are covered with a multitude of electrical discharge points in the form of hair, feathers, bristles, setae or spinules, or they have more electrically conductive skin (unlike that of snakes). Thus, snake skin appears adapted for acquiring and retaining static in the following ways: it is a good electrical insulator; a positive charge is produced by friction with nearly all common materials; and it has a relative absence of points where discharges may occur.

Second, the explanation that rattlesnakes avoid injury by warnings produced by noise of tail rattling¹ is partly correct, but harks back to times when this was the only explanation. The warning explanation becomes less convincing in the light of the facts that silent rattling is common in juveniles and smaller rattlesnake species, and that the primitive ancestral forms of the rattle were probably silent. Thus the rattle may have other functions unrelated to warnings.

We used the experimental arrangement shown in the figure to determine whether rattlesnake rattles could produce electrostatic charges solely by vibration in air. We attached a rattle (for example, from a western diamondback rattlesnake, *Crotalus atrox*) to an end of a coil of wire so that the rattle could be vibrated. The wire coil was held in place by acrylic plastic mounted over a static voltmeter (709 static sensor, 3M Co.). When vibrated at 60 Hz through an insulating nylon rod with no rattle in place, the voltmeter showed a zero voltage. But when the rattle was in place and vibrated, positive charges of 50–100 V were produced, indicating that a vibrating rattle generates electrostatic charges.

In feeding and in following trails, snakes touch objects and surfaces with



A rattle from a rattlesnake is attached to a wire coil; the wire coil is held by a strip of acrylic plastic over a static sensor voltmeter. When the rattle is vibrated at 60 Hz by lateral motion of the insulating nylon rod, a positive static charge of 75 to 100 volts is produced. When the nylon rod and coil are vibrated with no rattle in place, the voltmeter shows that no charge is produced.

their tongue tips and this is thought to assist the animal's olfactory and chemical sense².

In snake tongue scanning during exploratory activity, however, contact tends to be avoided as the tongue tips are waved overhead, forwards and down. Electrostatic charges present on active snakes would cause the scanning tongue tips to be repelled by other positive charges and attracted towards negative charges. Differences in environmental charge levels could be detected by proprioceptor or other sensory cells. The fact that moist air is conductive for the electric charges that exist on the Earth's surface could be very important to the snake's survival. The airborne plumes of moisture exhaled by animals and flowing out from under cover are invaded by static charges from the Earth and could be detected by snakes.

It will be difficult to test the hypothesis that snakes sense the common differences found in electrostatic voltages under natural conditions. Snakes remain motionless for long periods and, when active, they often respond poorly or not at all to rewards or negative reinforcements. We used time-lapse photography to record tests of one-week duration using stationary or daily changing levels and locations of charges. We replicated tests at random, substituting light intensities or odour concentrations in place of voltages (odour sources were small quantities of decomposing meat, moist humus, musks and dilute chemicals). These and other experiments demonstrated that snakes respond to laboratory levels of selected electrostatic voltages in much the same way as in

tests with levels of light and odour. Clearly, new ideas for tests under natural conditions are needed.

For the present, the hypothesis of an electrostatic sense in snakes interprets tail rattling and ancillary charging mechanisms as adaptations producing electrical energy for the distant detection of environmental features by snake tongue

scanning. Tongue scanning by snakes has been a long-standing puzzle which is not addressed in herpetology texts (for example, ref. 3).

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Fluctuating asymmetry in racehorses

SIR — Fluctuating asymmetry (FA), random fluctuations from perfect bilateral symmetry, has been used to study environmental deterioration^{1,2} and sexual selection³⁻⁷, but the relationship between FA and aspects of function in domesticated animals has been neglected. We show here that FA in thoroughbred racehorses has an effect on racing ability and may therefore be a predictor of future performance in young horses.

We measured 10 paired characters on 73 flat-racing thoroughbreds, four of which were on the forelegs of the horse and six on the head (see table). Relative asymmetry was calculated as right minus left, divided by the mean left and right character value. To eliminate inter-observer variation, all measurements were made by only one of us (L.O.). Characters were measured twice in ten individuals. Repeatabilities⁸ were high, ranging from 0.97 to 1.0. Hind legs were not measured because movement tended to reduce repeatability.

The abilities of racehorses are estimated by handicappers. The official ratings⁹ provide one such estimate of racing ability. Horses start their racing career as two-year-olds and their rating is modified after each race. Ratings are therefore retrospective and cannot be used for yearlings. The table shows the correlation between ratings and FA for all

10 characters and overall mean FA. The relationships are all negative: that is, symmetrical horses have higher ratings than asymmetrical animals particularly for features of the head. The correlation is strongest for overall mean FA (which may reflect individual viability) and asymmetry (see figure). Horses are also handicapped on the basis of age. Three-year-olds receive a higher weight handicap than two-year-olds, and four-year-olds a higher weight than three-year-olds. The effect of adding the weight-for-age handicap is shown in the table for five furlongs, 32 lb being added to horses of three years old or more, and 13 lb to horses of four years old or more. FA is now, in general, more closely correlated to rating plus weight allowance than to rating alone.

This finding suggests that FA is negatively correlated to age, and this is indeed the case ($r = -0.38$, $F = 12.01$, $P = 0.0009$). But a multiple regression, with rating as the dependent variable and FA and age as independent variables, shows that FA and age are independently associated with rating (FA:rating $r = -0.34$, $F = 10.03$, $P = 0.002$; age:rating $r = -0.28$, $F = 6.59$, $P = 0.012$).

At present judging young racehorses for future performance is an uncertain process. Heritability for speed has been estimated as ranging from 5 to 55 per cent¹⁰ and the performance of the dam

PRODUCT-MOMENT CORRELATIONS (r) BETWEEN FA OF CHARACTERS AND OVERALL MEAN FA AND RATINGS OR RATINGS PLUS AGE ALLOWANCE

Character†	r (ratings)	P	r (ratings + age all)	P
Elbow-knee	-0.15	0.19	-0.15	0.21
Knee-ergot	-0.05	0.65	-0.06	0.64
Knee thickness	-0.15	0.21	-0.24	0.03
Coronet band	-0.19	0.11	-0.22	0.06
Ear height	-0.10	0.40	-0.02	0.89
Teeth height*	-0.21	0.07	-0.38	0.001
Teeth width*	-0.12	0.31	-0.14	0.24
Nostril width	-0.22	0.06	-0.20	0.09
Cheekbone-ear	-0.04	0.72	-0.06	0.59
Cheekbone-mouth	-0.35	0.0027	-0.33	0.0047
Overall mean FA	-0.43	0.0002	-0.48	0.0001

All relationships are negative; that is, symmetrical horses are rated more highly than asymmetrical. Surprisingly, this is particularly true of the head characters. The relationship between the FA of cheekbone-corner of mouth and rating provides the strongest correlation for individual characters. FA of the head may reflect general viability in addition to sensory advantages in judgement during racing. The overall mean FA provides the best estimate of racing ability.

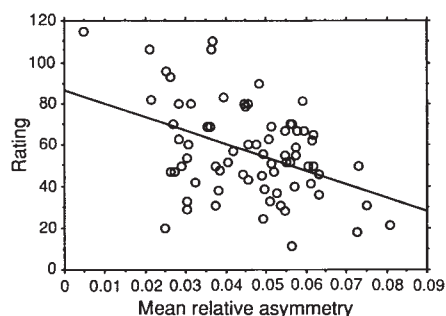
*Measurements were made on the first pair of upper incisors.

†Callipers and a tape measure were used to an accuracy of 0.1 mm and 5 mm respectively.

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Relationship between the racing ability of 73 thoroughbred racehorses as measured by the ratings of the British Horseracing Board and overall mean FA per horse ($y = -653.49x + 86.87$, $r^2 = 0.18$, $r = 0.43$, $F = 15.75$, $P = 0.0002$).

and sire is the most reliable predictor of racing ability. Estimates of overall FA may provide an additional tool which could be used, with due correction for age, in the process of choosing future good performers on the track.

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Born too late to win?

SIR — Dudink reported¹ a skew in the distribution of birth dates of professional soccer players in England and Holland, suggesting that younger children in any age group participating in sporting activity may be disadvantaged. But from these data alone it is impossible to tell whether the sources of this bias are psychological in origin² or based on physical advantages.

To examine this question, I have collected birth dates and heights of county cricketers in the United Kingdom for the 1991 season³. Because the season starts in April, I analysed birth dates in six-month blocks (April–September and October–March). A birth-date effect was found for fast bowlers (57 early-year bowlers versus 36 late-year bowlers; $\chi^2(1)=4.30$, $P<0.05$) but not for spin bowlers (20

versus 17), batsmen (63 versus 64) or wicketkeepers (17 versus 18; in each case, $\chi^2(1)<1$, $P>0.05$). Because cognitive development is presumably equally advanced in all four categories of cricketers, it seems likely that physical rather than psychological factors may explain the uneven effect. However, as spin bowlers, batsmen and fast bowlers are significantly taller than the population mean of 1.78 m ($P<0.0001$ in each case), it is unlikely that height advantage alone can explain the birth-date effect in fast bowlers.

A closer analysis of the data concerning soccer players for the season 1990–91 (ref. 4) shows that the birth-date effect is true for goalkeepers, defenders, midfield players and forwards (at least $P<0.05$ in each case). But the average height of goalkeepers and defenders is significantly greater than that of the general population ($P<0.0001$ in each case); midfield players are significantly shorter ($P<0.0001$) and forwards conform to the average.

Thus, in cricket, only fast bowlers show a birth-date effect, even though spin bowlers and batsmen are also significantly taller than the average. In football, all categories of players show the birth-date effect, even though only goalkeepers and defenders are taller than average. Thus, height advantage alone cannot account for the birth-date effect in these sports. Because advantage in psychological development is presumably uniform across categories of players in the two sports, and because not all the categories of players show the birth-date effect, advantage in psychological development alone cannot account for the effect either. Thus, success in certain sports must be influenced by some other physical attribute, such as strength, or different sports may demand different combinations of psychological and physical attributes.

Whichever explanation is correct, these data suggest that one strategy to overcome the disadvantage conferred upon some individuals would be simply to encourage the appropriate individuals into the appropriate sports during childhood. Such a strategy might minimize the misery experienced by those forced to take part in inappropriate sports, while maximizing the chances of producing sporting excellence across a range of sports.

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■ Editor's note: J. Verhulst has written to point out that he published a paper reporting similar conclusions to ref. 1 in *Medical Hypotheses* **38**, 346–348; 1992.

SIR — Unfortunately, Dudink's concerns¹ about the influence of birth date on talent selection seem only too true. Young athletes in certain sports, whose birth dates fall during the early part of their sports selection year, are more likely to be identified as 'talented' than those born later^{2,3}. This suggests that current talent identification is significantly influenced by a child's physical attributes rather than his or her sporting ability.

Our data come from a longitudinal study of the effects of intensive training on a child's psychological and physiological development⁴ covering 1987 to 1992. In brief, 453 athletes aged 8–16 years (222 female athletes from gymnastics, swimming and tennis, and 231 male athletes from gymnastics, swimming, tennis and soccer) were drawn from a 300-mile radius of London. All athletes were 'élite' for their age according to criteria provided by each sport's governing body.

In most sports, 1 January is the start of the selection year. When we examined the distribution of birth dates of the athletes studied we found no differences between genders in any sport. But almost half the tennis players and swimmers were born within the first 3 months of the year, with a gradual decrease thereafter. Most soccer players were born in the last half of the year; this is the first half of this sport's selection year. As late physical development and sexual maturation appear to favour potential élite gymnasts⁵, we were surprised to find a uniform distribution of birth dates throughout the year in this sport. (Detailed figures available from the authors on request.)

The relationship between date of birth and sporting success, particularly in sports where advanced physical development is advantageous, implies that the youngest children (biological and chronological) in any age grouping are at a considerable disadvantage. The fault lies in age-banded training and competition. If talent selection in sports such as tennis and soccer is indeed based on physical attributes, many talented individuals may be being overlooked simply because they are born late in the selection year.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. Priority will be given to letters of fewer than 500 words and five references.

Food from heaven

Roy Porter

From Fasting Saints to Anorexic Girls. The History of Self-Starvation. By Walter Vandereycken and Ron van Deth. *Athlone/New York University Press: 1994. Pp. 296. £18.95, \$42.50 (hbk); \$17.95 (pbk).*

THOSE seeking historical perspectives on the present epidemic of eating disorders have been well-served by recent historians — the books of Joan Jacobs Brumberg, Rudolph Bell and Hillel Schwartz come especially to mind. This study by two Dutch psychiatrists is a valuable addition to attempts to elucidate a tragic problem.

Choosing the wide-angle lens — their study proceeds from Antiquity to the present, covering the Old World and the New — Vandereycken and van Deth have wisely avoided the reductionist trap of medicalizing the past. Although noting the antiquity of the medical term 'anorexia' (literally, absence of appetite), they disclaim the psychiatrically tempting supposition that all self-starvers in the past have been afflicted by a clinical disorder — in other words, they might be retrospectively diagnosed as anorexic. Instead, they strive to lay bare the meanings of earlier self-starving behaviour, while exploring the advent of anorexia nervosa, that is, the moment at which strange eating habits fell under medical and, above all, psychiatric scrutiny.

The authors propose a three-stage history. Early self-starvation was characteristically conducted within a religious agenda whose goal was the mortification of the flesh. All Christians were to renounce the deadly sin of gluttony and to fast as directed by the Church. But Desert Fathers and hermits pursued food asceticism on an heroic scale, undergoing prolonged fasting or supping on nothing but locusts and grass; some female saints pursued particularly pious food preferences, refusing all food but the Host.

While perfecting holy abstinence, mediaeval Catholicism recognized its perils: fasting unto death was the ultimate sin of suicide, and in any case such austerities might not be piety but vanity or even the promptings of the devil. It comes as no surprise, therefore, that Protestantism was vigorously dismissive of such superstitious balderdash, and, partly for that reason, the era of spiritual fasting gave way to a period of fascination with starvation as spectacle. The eighteenth and nineteenth centuries promoted the faster as freak, a local curiosity for ghouls to swarm around or an object of commercial

exploitation alongside midgets and bearded ladies in fairs and circuses. Protégés of P. T. Barnum achieved great celebrity as hunger artists, staging grand public fasts in theatres before a voyeuristic public, titillated by such feats of self-denial and macabre skeletal displays.

It was at this stage that the involvement of the medical profession intensified.



The thirteenth-century Saint Hedwig of Silesia, who dieted solely on fish, bread, vegetables, milk and water (1504).

Occasionally physicians would authenticate these great exhibitions of emaciation, but most often they intervened as sceptics, vociferously denying the physiological possibility that marvellous maids might survive for years on nothing but sips of whey, or even the air, and so serving as fraud-busters.

But this inescapably led to the third stage. For when these public-spirited exposés failed to put a salutary end to the fasting fad, the doctors had no alternative but to pronounce the whole palaver not, after all, just an arrant swindle but a sickness in its own right. The self-starvers (characteristically female) were not so much cheats as ill — sick in body but above all in mind. Initially the label affixed with gay abandon from early Victorian times was 'hysterical'; hysteria had, after all, become the portmanteau diagnosis for female perversities of the will and expressions of social and sexual

deviance. But from the 1850s, more specialized psychiatric terms began to be coined: *anorexie nerveuse*, *anorexie gastrique* and so on.

As is well known, a priority dispute erupted around 1870 between (Sir) William Gull and the French neuropsychiatrist, Ernest Lasègue, as to who had first deployed the term *anorexia nervosa* in its full clinical sense. Vandereycken and van Deth incline to Lasègue, but insist that such priority questions are trivial matters in contrast to the telling fact that from around the 1870s the historian can at last without anachronism speak about the presence of the anorexia syndrome. In other words, by then there were not merely fasting people, but self-starvers with personality dispositions recognizably akin to the classic anorectics of the post-war period.

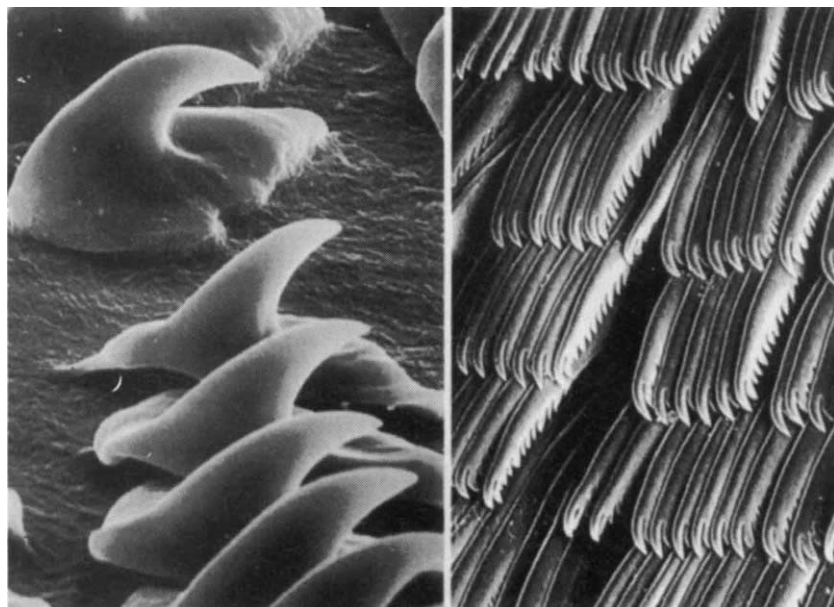
And herein lies the conceptual thrust of this intelligently argued (and admirably translated) work. It is not helpful, the authors claim, to label ancient self-starvers 'anorectics' because their mindsets and behaviours were essentially dissimilar from the modern paradigm: in the Middle Ages at least, fasting was an act of orthodox piety not of personal rebellion. Above all, 'slimness' was a goal unknown before emaciation and emancipation became linked in modern feminism.

So Vandereycken and van Deth's contention is that although anorexia nervosa is indeed a psychiatric disorder, it is one that cannot be parcelled up in a reductionist manner, isolated from the wider social systems and cultural beliefs that provide its roots and afford its meanings. Moreover, unlike some recent feminist writers, they maintain that no rivalry

should be assumed between the 'disease' and the 'protest' models. Rather, modern anorexia is the biopsychosocial disorder that mirrors a society with tensions and contradictions: the bourgeois family, supportive but suffocating, and all the paradoxical hypocrisies of attitudes towards youth, food, beauty and sexuality, exploited by the media and affluence industries.

And even as we begin to grasp anorexia, it may be fading away in front of our very eyes, transforming itself in a protean manner into new and hitherto little understood disorders of consumer society. Meanwhile, Vandereycken and van Deth have contributed not a little to our comprehension, and deserve our thanks for a well-researched work that shows how history and psychiatry may be allies. □

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THE cutting edge — chitinous teeth of the radula, the unique tongue-like feeding organ of molluscs that works in much the same way as a rasp and conveyor belt. The teeth displayed here are from the marine gastropods *Berthella stellata* (left, $\times \sim 30,400$) and *Berthellina citrina* (right, $\times \sim 4,000$). From *Microscopic Anatomy of Invertebrates*, Vol. 5: *Mollusca I* edited by F. W. Harrison and A. J. Kohn (Wiley, \$213, £152).

An American tale

Stuart L. Pimm

A History of the Ecosystem Concept in Ecology: More than the Sum of the Parts.

By Frank Benjamin Golley. Yale University Press: 1993. Pp. 254. £25, \$30.

I WAS once denied a grant because I was "not an ecosystem ecologist". Frank Golley tells me why: I am the wrong nationality. Despite the British invention of 'ecosystem' and its first-place ranking among concepts by contemporary British ecologists, "the ecosystem story is largely an American tale". Golley tells how ecologists with a common language have trodden separate paths from the start.

Golley is in a unique position to do this. He and his colleagues in Georgia have been key players in ecology for nearly 40 years, but friendships and personal investment have not produced an eulogy. The book's strength is its detached evaluation of researchers and some of ecology's largest research programmes. Readers will find an excellent account of how crucial ideas developed and will ponder how much a lifetime's work can accomplish.

The history begins with Arthur Tansley, who coined the term 'ecosystem' in 1935, and his discussions with John Phillips; moves on to Raymond Lindeman, the brothers Odum, the International Biological Program (IBP)'s "Analysis of Ecosystems" project and George van Dyne;

and finishes at the end of the 1970s.

The tale becomes a largely American one with Lindeman. The initial rejection in 1941 of his paper "The trophic dynamic aspect of ecology" is a familiar story. One reviewer (probably Chancey Juday, a distinguished limnologist of the day) wrote: "[most of] the . . . discussion and argument is based on belief, probability, possibility, assumption, and imaginary lakes, rather than on actual observation and data." That comment was prescient. Sadly, Golley does not contrast Lindeman's concerns with those of contemporary British ecologists. They too were grappling with the ecology of things more complex than populations. At a meeting in 1944, Charles Elton was counting the number of species per genus in plant and animal communities and David Lack was measuring beak dimensions of Darwin's finches.

Golley writes of the next two decades: "that the ecosystem concepts were not presented as hypotheses to be tested or questions to be asked was the most serious weakness . . . of this period". Observations test hypotheses. Yet observations were no more in evidence in 1965 than when Lindeman's paper was rejected in 1941. Up to here, the middle of the book, there are only two graphs or tables with numbers in them (one is Golley's, the other Juday's). An example of this ecologists' never-never land is Eugene Odum's assertion in 1959 that as ecosystems developed over time they would become dynamically stable. One could explore this hypothesis through both computer modelling and empirical testing. In fact,

Odum stated his idea as a principle and not a hypothesis, which inhibited testing. A Brookhaven workshop in 1969 explored the principle and found it wanting. In neither Odum's proposal nor the meeting's proceedings is there a single graph or table of empirical or theoretical results with stability on one axis and time (or anything else) on the other.

The IBP was to change all this. How quickly it lost direction! Initial ideas stressed the measurement of "organic production and decomposition at different trophic levels". In 1965, Eugene Odum chaired the terrestrial productivity group. The themes developed in cooperation with the freshwater group were production, trophic structure, energy flow, nutrient cycling and the like on a planetary scale. By 1967, American modellers had taken over and the language was "driving forces", "components" and "coupling". The grassland model was ELM — for Intermediate Level Model. Neither the inability to spell nor the awful pun in the naming of this model of a treeless system humoured the field biologists. We lowly graduate students were charged with collecting the data to validate (not test) the models. Favoured faculty members studied the few processes that the modellers did not fully understand. The model was supreme and its triumphant presentation on the last day of each annual meeting was our reward. By 1974, a new catch-phrase buzzed in the Fort Collins bars on the first evening: "non-modelling synthesis". The data, their synthesis and analysis might be interesting in their own right. Golley's evaluation of the IBP is short, honest and brutal. He does not tell us that for a long time after the IBP, the only accessible compilations of data on ecosystems were those compiled before the project.

Golley's final thoughts are also interesting, for his story provides the grist for a powerful mill: comparative science. The developments in other countries tell us much about why they followed different paths. Elsewhere, he correctly emphasizes how the desire for clear hypotheses and their testing through experiment and observation led researchers away from ecosystem studies. Golley also suggests a British disdain for large research teams and their delight in the individual project. More crucially, he makes a compelling case for the need for the comparative data that only large teams can provide. Ecosystem ecology for too long has operated in a dream world with few hypotheses and even fewer data. The clear lessons from this book are that for ecosystems we will win neither the hypotheses nor the data easily. □

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Why is anything ever simple?

John L. Casti

The Collapse of Chaos: Discovering Simplicity in a Complex World. By Jack Cohen and Ian Stewart. Viking: 1994. Pp. 496. £18, \$23.95.

SOMEONE once described Los Angeles as 38 suburbs in search of a city. I can't think of a better metaphor to describe how I felt as I began my trek through this book. For the first 200 pages or so, I tried valiantly not to be distracted by the fineries of viruses, information theory, reductionism, algorithmic complexity, the genetic code, the Game of Life, Langton's ant, Lorenz's butterfly, dialogues with alien scientists and the dozens of other intellectual goodies put on the table before me. Not once tempted by these blandishments, I steadfastly persevered in search of the city surrounded by these suburbs of the intellect. And, as they say, all things come to those who wait — provided that they're willing to wait long enough — and on page 222 I finally found the Holy Grail. The authors' mission, it turns out, is to convince the world that scientists have been focusing on the complexities of a system when what really needs explaining is its simplicities. In short, if there's all that complexity out there, why is anything ever simple? *Voilà*. From there on it was as if I were in a hot-air balloon soaring over the landscape of complex systems, being continually regaled by tales of the world below by guides intimately familiar with every nook and cranny of the territory.

The "collapse of chaos" in the title refers to the way in which complex behaviour emerging from simple rules at one level can itself give way to simple behaviour at another hierarchical level. Although one might quibble with the conflation of chaos and complexity, this process of the emergence of simple properties and patterns from complex behaviour is the key in the rapidly unfolding science of complex systems, so the authors have certainly chosen well to have it constitute the centrepiece of the second half of the book.

The authors draw a useful distinction between reductionism as a way of getting at the scheme of things and emergent behaviour, regarding the first as focusing on the inside of systems, their content, so to speak, and the latter emphasizing that which lies outside the system, its context. These are essentially dual aspects of any system, which strongly suggests that both are needed to understand fully the whys and wherefores of any complex process. Yet conventional science focuses almost exclusively on the system's context, which seems to be a bit like trying to

play basketball with one arm tied behind your back. It can be done, but you'll certainly do better with both arms engaged in the action. In fact, the book argues that it's flatly impossible to understand emergent phenomena if you don't look at both sides of this duality and consider the system's interactions with its environment.

To characterize emergent behaviour, the authors revive two archaic words, "simplicity" and "complicity", which to many readers (including this reviewer) may at first glance appear to be all-too-cute neologisms. But there turns out to be considerable merit to this semantic trick. Simplicity is related to the word 'simplex', meaning simple, and refers to 'regular' emergence, the kind whereby a system of rules gives rise to simple features. A good example is the appearance of the celebrated Feigenbaum constant in all period-doubling routes to chaotic behaviour. Complicity, on the other hand, is a kind of 'super' emergence, in which completely different rules converge to produce similar features. The authors cite consciousness and evolution as prime examples of this kind of emergence. So simplicity explores a fixed space of the possible, whereas complicity enlarges the space. Regardless of the appellations, these are definitely useful distinctions to draw about the behaviour of complex systems, and it is to the authors' credit that they present them in such an easy-to-digest fashion for the general reader, not to mention the scientific public.

Despite the intimidating theme, which may suggest to many a fairly dull tramp along several well-worn paths in the philosophy of science, I think most readers will be pleasantly surprised to find in this work a host of new and thought-provoking ideas about the workings of complex systems. The authors have taken considerable pains to describe their ideas in familiar terms, with many examples — mostly from biology — and have admirably made the point that something fundamental is missing in the traditional reductionist view of science.

Simplicity and complicity may or may not make their way into the scientist's lexicon, but the ideas they encapsulate about system complexity and simplicity will almost surely form the backbone of twenty-first-century science. And this book is as good an introduction to that science of the future as any you're likely to find. □

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New in paperback

More than 70 scholars worldwide contribute to **The Cambridge Encyclopedia of Human Evolution** edited by Steve Jones, Robert Martin and David Pilbeam (Cambridge University Press, £24.95). "A feast of information. . . If (whether student or professional) you're interested in human evolution, you'll want to have this volume handy" (Ian Tattersall, *Nature* **361**, 314; 1993).

A comprehensive review of ageing is provided in Caleb E. Finch's **Longevity, Senescence, and the Genome** (University of Chicago Press, £35.95), now published with expanded and updated references and bibliography. "A treasure house of fascinating information . . . which will undoubtedly pave the way for comparative work" (Linda Partridge, *Nature* **351**, 198; 1991).

The basics of immunology are elegantly summarized in **Life, Death and the Immune System** (W. H. Freeman, £13.95). Written by respected workers in the field, the chapters originally appeared as articles in the September 1993 issue of *Scientific American*.

Princeton University Press has reissued two books in its Science Library series intended to bring the writings of leading scientists to a broad professional and general audience. They are: **The Enjoyment of Math** by Hans Rademacher and Otto Toeplitz, first published in 1957, a selection of mathematical problems and their solutions; and **The Supernova Story** by Laurence A. Marshall, which was described as "a superb piece of writing [which] reads almost as easily as a good novel" (H. Shipman, *Nature* **336**, 291; 1988). Each is priced at \$12.95, £10.95.

Stress Test (Rutgers University Press, \$10.95) is a collection of cartoons on medicine by the irrepressible Sidney Harris. An example of his humour appears below.



"YOU CAN TELL DR. RIDLEY THAT I KNOW KLEINZOFF'S SYNDROME WHEN I SEE IT, AND YOU DON'T HAVE KLEINZOFF'S SYNDROME."

In the beginning

John Gerhart

Embryos: Color Atlas of Development. Edited by Jonathan B. L. Bard. Wolfe: 1994. Pp. 224. £49.95.

As Jonathan Bard explains in his introduction, the discovery of a host of molecules shared by a wide variety of developing organisms has been the first reward for those analysing the molecular genetics of developmental processes. This has stimulated interest in comparative development as an inquiry into the shared molecular mechanisms of development. This book was prepared to aid such studies, not by summarizing the results of molecular developmental research but by providing background information on the development of 12 organisms commonly used in research, each covered in a chapter by an author well known for research in the field: *Arabidopsis* (the single plant example), *Dictyostelium* (a slime mould, not an embryo), sea urchin, nematode worm (*Caenorhabditis elegans*), molluscs, leech (annelid), *Drosophila* (insect), zebrafish, *Xenopus* and other amphibia, chick, mouse and human. The text of each chapter contains a description of the stages of development; an outline of advantages the organism holds for experimental studies, such as the analysis of mutants blocked in various aspects of development; a brief mention of the status of molecular analysis; a forecast of the organism's place in future developmental studies; and key references. All chapters are well illustrated by colour plates and diagrams (600 colour plates in total).

This is a valuable compendium for all students of development. Undergraduate or graduate students can use it with a separate text, and researchers can use it with texts and research articles. Many of the illustrations are valuable for teaching. The colour plates are of high quality, and the authors have made an effort to collect excellent material from their research colleagues and to present their own photographs. It is a pleasure just to leaf through the book and look at the pictures.

Beyond increasing comprehension, colour figures make some central phenomena of development inescapably apparent in ways they weren't before. All readers will be struck by region-specific gene expression as revealed by the new staining techniques involving *in situ* hybridization, *lacZ* reporter constructs and multicolour antibodies. A set of colour plates of regional gene expression at successive stages of an embryo's development reveals a strikingly dynamic and complex anatomy, one hardly appreciable by simply looking down a microscope. Still the wellspring of regionally expressed

test molecules, *Drosophila* provides the most impressive pictures, but the techniques have spread to other organisms, such as nematodes, sea urchins and recently amphibia and mice. It is to be hoped that a second edition of the atlas will follow in a few years when comparable *in situ* and antibody pictures are available for these organisms as well. □

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Geo-exploration

David Gubbins

Earth Sounding Analysis: Processing versus Inversion. By Jon F. Claerbout. Blackwell Scientific: 1992. Pp. 304. £45. Latest CD-ROM version available from J. F. Claerbout, Department of Geophysics, Stanford University, Stanford, California 94305-2215, USA.

THE Hitch Hiker's Guide to the Galaxy is a fictional electronic book. It resembles a notebook computer, provides useful information for galactic travellers and has DON'T PANIC on the front. *Earth Sounding Analysis* is a real electronic book: in addition to a paper edition published some time ago, there are more recent CD versions that promise interactive diagrams, machine-readable text and computer programs. It is an imaginative step forward in scientific publishing, despite being a highly specialist work.

Claerbout is an innovative leader in seismic-processing research, through the Stanford Exploration Project. His style is pleasantly casual. The first four chapters give essential background mathematics and the remaining chapters concentrate on seismic-processing methods. Relegating statistics to the final chapters will bother statisticians but is probably how seismic processing should be taught.

The material is difficult but rewarding. Particularly useful are the conventional discussion of the conjugate gradient methods, the innovative and intriguing treatment of missing data and the chapter on minimum phase. I did not fully understand the chapter on conjugate operators. Although this subject comprises an important underlying philosophy, it needs more mathematical development and structure. Claerbout, like most exploration geophysicists, has a very narrow definition of 'inversion' — least-squares modelling. Perhaps for this reason or perhaps because I did not understand the message on conjugate operators, the 'versus' between inversion and processing is not obvious. I expected a contrast between optimization and filtering methods, but the only clear

contrast in the book is between practical and impractical methods.

The electronic version contains both the book and PhD projects. Claerbout clearly believes his product goes beyond simply electronic reproduction of text and computer programs; it can be used to reproduce the calculations done during the research, to rebuild datasets and programs and to extend applications to new problems. He uses the term "reproducible research", by which he means the publication of working programs and data as an integral part of a scientific report, made possible by the electronic revolution. It is different from 'reproducibility of science', where a scientific result is described in sufficient detail and with sufficient accuracy for it to be repeated by others.

For example, scientific reproducibility allows us to test Newton's law of gravitation to far greater accuracy than could seventeenth-century astronomers. The result is strengthened if confirmed, or gives way to a new theory (general relativity in this case) if some discrepancy is found. This scientific process is different from repeating Tycho Brahe's observations with the same instrumentation: we might want to do this to clarify our understanding of the original procedures, but the results would add nothing to science. Claerbout is concerned with this latter, more practical goal: a worthy one in view of the lax nature of many modern scientific papers and the dependence of the results on bug-prone computer programs.

As well as the CD-ROM drive, the electronic book needs 15 megabytes of hard-disk space and creates hundreds of irritating files. It gives access to all the text and an enormous volume of exploration geophysics software. Few of the diagrams are interactive, and those that are depend on just two different programs. Unfortunately, the CD cannot be used over the network, and it took a long time on our machines (Sun 4) to draw any of the diagrams.

At Leeds we teach seismic processing and inversion using a program called XPITSA, available through the International Association of Seismology and Physics of the Earth's Interior. It is menu-driven (and therefore quick for students to learn), occupies little disk space, and encompasses far more of the subject than the interactive diagrams in Claerbout's book.

Claerbout's important contribution to the electronic publishing revolution is to put together publications with programs and data, as an essential new type of scientific publication. Not quite *The Hitch Hiker's Guide*, perhaps, but a big change in the way we learn and do science. □

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Vesicle fusion from yeast to man

Susan Ferro-Novick & Reinhard Jahn

Membrane budding and fusion occur in all eukaryotic cells. Their underlying mechanisms have been studied in mammalian neurons and in yeast, a simple eukaryote. The differences between these two systems would suggest that fusion events in yeast and the neuron would operate by different mechanisms, but recent advances indicate that this is not true.

SUPERFICIALLY, protein transport in yeast and synaptic vesicle release from nerve endings appear to be very different. Secretion in yeast is a constitutive process, for example, whereas synaptic vesicle exocytosis is tightly regulated by intracellular Ca^{2+} concentration. Furthermore, once exocytosis is activated in the nerve terminal it proceeds faster than in other secretory systems, with a time lag of only 200–300 μs . Given these differences, it was reasonable to assume that the two processes would occur by different mechanisms mediated by unrelated proteins, just as different enveloped viruses have developed structurally unrelated proteins that are fusogenic¹. Recent advances, however, indicate that this is not the case.

The 'SNARE' hypothesis

The terminal membrane proteins synaptobrevin (also known as VAMP), syntaxin and the synaptosomal associated protein of relative molecular mass 25,000 (M_r 25K; SNAP-25) are structurally related to 10 yeast genes many of which are required for the targeting and/or fusion of transport vesicles with their acceptor compartment at different stages of the secretory pathway^{2–5}. Two independent approaches provide strong evidence that these nerve terminal proteins are directly involved in neuronal exocytosis. First, they are selectively proteolysed by tetanus and botulinum toxins, powerful inhibitors of neuronal exocytosis^{6–10}. Second, they interact with the soluble proteins *N*-ethylmaleimide-sensitive factor (NSF), and α - and γ -soluble NSF attachment proteins (SNAPs) which are necessary for intracellular fusion¹¹. Although these proteins were originally purified on the basis of their ability to restore activity to a mammalian intra-Golgi vesicular transport assay^{12,13}, homologous yeast components can replace the mammalian proteins in this assay^{14,15}. The yeast homologue of NSF is the *SEC18* gene product, whereas *SEC17* encodes α -SNAP^{14,16}. *SEC18* (NSF) is required for transport at multiple stages of the secretory pathway¹⁷. Thus, intracellular membrane fusion events in all eukaryotes appear to involve similar mechanisms using identical or related components. The discoveries that led to the identification of these components are discussed below.

Our current concept of membrane fusion is based primarily on the interactions between soluble fusion factors and their receptors. NSF, a homotetramer, binds to membranes when one or more of the SNAPs are bound to their receptors (SNAP receptors, 'SNAREs'). In neurons, this interaction produces a multi-subunit complex which can be extracted as a 20S particle containing the membrane proteins synaptobrevin, syntaxin and SNAP-25¹¹. These receptors form a complex before the soluble factors bind¹⁸. On the basis of these observations, Rothman and his colleagues proposed that an integral membrane protein resident on the transport vesicle (the v-SNARE, synaptobrevin in nerve terminals; see Fig. 1) pairs with its receptor on the target membrane (the t-SNARE, syntaxin in nerve terminals) to initiate fusion. The t-SNARE, which is probably complexed with another membrane-bound protein (t-SNARE 'assistant', SNAP-25 in nerve terminals), interacts only with its matching v-SNARE, ensuring that the transport vesicle fuses with the correct acceptor compartment. The 20S particle is disassembled

when NSF cleaves ATP, an event thought to precede membrane fusion¹⁸. The v-SNARE does not accumulate on the target membrane, suggesting that it is recycled after fusion.

According to the 'SNARE hypothesis'^{11,18}, each form of intracellular fusion has its own set of specific v-SNAREs and t-SNAREs, whereas NSF and SNAPs are common to all forms. Non-neuronal relatives of nerve terminal proteins indeed participate in fusion events at different steps of the trafficking pathway in yeast (see Fig. 1). The fusion events involved in membrane recycling may, however, be somewhat different. Synaptic vesicles, for example, fuse with the plasma membrane during exocytosis and with early endosomes after endocytosis (for a review see ref. 19). Fusion with endosomes is dependent on NSF and α -SNAP²⁰, but does it also involve the exocytotic v-SNARE synaptobrevin? Neither the rate nor the extent of endosome fusion is affected when cellubrevin, a non-neuronal homologue of synaptobrevin, is degraded in BHK cells²¹. Thus, a separate set of membrane proteins may therefore be needed for recycling events. Three putative v-SNAREs (Boslp, Sec22p and Betlp, see below) have been implicated in endoplasmic reticulum to Golgi transport in yeast, and at least one of these proteins may play a role in retrograde transport.

Yeast genes encoding neuronal homologues

How were the yeast proteins essential for membrane traffic identified? The key has been the isolation of lethal mutants that block cell surface expansion (for a recent review see ref. 22). Many of these proteins have been identified from temperature-sensitive (ts) mutants which die at 37 °C, but not at 25 °C. Others have been isolated on the basis of their ability to compensate for a defect in membrane traffic that would otherwise result in cell death when overexpressed. Such genes usually encode proteins with a function related to the suppressed defect. The final proof that the identified gene product is required for secretion is the demonstration that protein transport is blocked in its absence.

Although Schekman and his colleagues began a classical genetic approach more than 14 years ago to isolate the genes that encode such proteins^{23,24}, many have nevertheless been isolated as high-copy suppressors. This is partly because several of these genes are redundant (such as *SNC1/SNC2* and *SSO1/SSO2*) and phenotypes are only apparent when both genes are deleted^{3,4}. In addition, the original selection only identified a fraction of the genes required for vesicular transport, and many genes were identified by the suppressor approach before further screens for mutants could be done.

Apart from the *unc*-mutants in the nematode *Caenorhabditis elegans* (reviewed in ref. 25), many of which are deficient in neurotransmission, no comparable mutant collection is available for the study of neuronal exocytosis. Most neuronal trafficking proteins were therefore identified by characterizing synaptic vesicle proteins¹⁹. Synaptic vesicles are easy to purify in large quantities, aiding analysis of this organelle. Obviously, this approach only identifies proteins involved in synaptic vesicle exocytosis and recycling. Not surprisingly, these proteins are generally abundant in synapses and are specific for neurons and neuro-

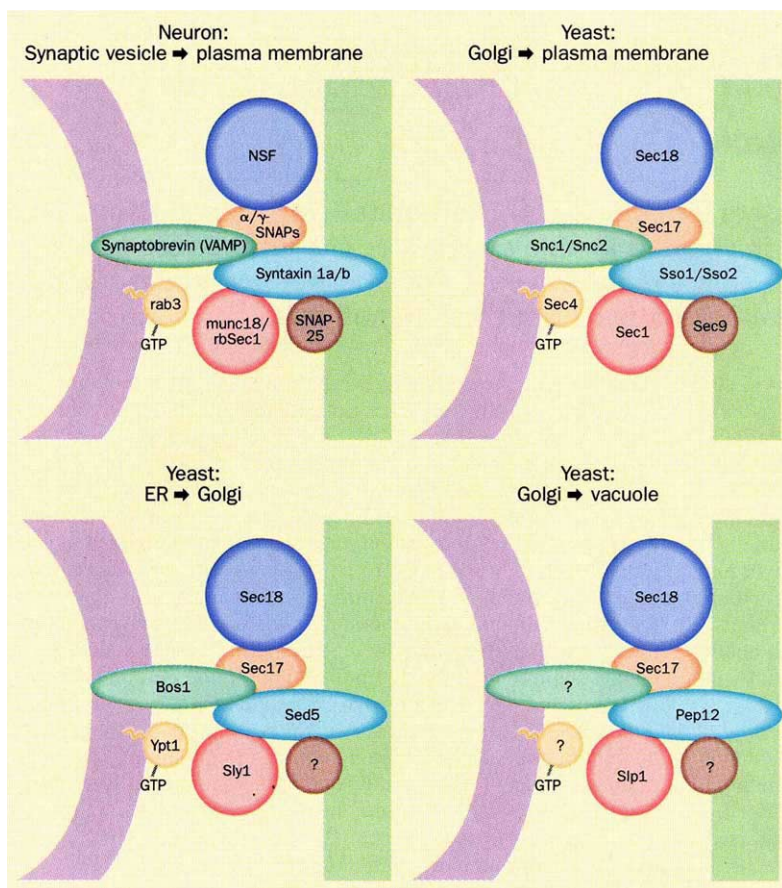


FIG. 1 Conserved components that function in vesicle targeting and fusion from yeast to man. The model shown for the neuron in the top left panel is based on protein-protein interactions that were demonstrated by biochemical studies. The remaining panels show the homologues of these proteins that function at different stages of the secretory pathway in yeast. The interactions depicted for the yeast gene products are largely based on genetic studies. These studies have provided the *in vivo* correlate for this model. In some cases, the *in vivo* experiments have been corroborated by *in vitro* studies of membrane transport.

endocrine cells¹⁹. Isoforms of synaptobrevin and syntaxin are present in many non-neuronal cell types in mammals^{26,27}, but the trafficking steps in which they act are unclear. The only known non-neuronal form of synaptobrevin, cellubrevin, probably functions in post-Golgi transport²⁶.

Synaptobrevin

Synaptobrevin is an 18K integral membrane protein anchored to the exterior of synaptic vesicles by a single carboxy-terminal transmembrane domain (reviewed in ref. 19). In yeast, five genes (*BOS1*, *BET1*, *SEC22*, *SNC1* and *SNC2*) encoding proteins

related to synaptobrevin have been isolated (Table 1). The most closely related is *Snc1*, which is about 40% identical to synaptobrevin²⁸. *Snc2*, the gene for which was cloned on the basis of its homology to *Snc1*³, is 79% identical to *Snc1* and 32% identical to synaptobrevin. Both proteins are located in post-Golgi transport vesicles³. Yeast strains lacking *SNC1* or *SNC2* have no apparent phenotype^{3,28}, but strains lacking both have defects in post-Golgi secretion³, demonstrating that these proteins are required for exocytosis.

Other proteins related to synaptobrevin function at earlier stages of secretion. *BOS1*, a gene identified by its ability to suppress the secretory mutants *bet1* and *sec22* (ref. 29), was the first relative of neuronal proteins shown to be required for vesicular transport in yeast². *Bos1* is 27% identical to *Snc1* (Y. Jiang, J. P. Lian and S.F.-N., unpublished observations), and like synaptobrevin is an abundant constituent of transport vesicles^{5,30}. In an *in vitro* reaction reconstituting endoplasmic reticulum to Golgi transport⁵, anti-*Bos1* antibody blocks vesicle fusion, but not budding. Furthermore, vesicles formed by cells lacking *Bos1* fail to fuse with the Golgi, whereas cytosolic and Golgi fractions from these cells support transport. Although *Bos1* primarily resides on the endoplasmic reticulum membrane, its depletion from this compartment therefore only blocks the activity of the transport vesicles, indicating that it is a key component of the targeting/fusion machinery on endoplasmic reticulum to Golgi vesicles. Because *Bos1* is not found on the Golgi apparatus, it is either degraded or recycled after vesicle fusion³⁰. *BOS1* also interacts genetically with *BET1* and *SEC22*, two other genes whose products have the same topology as *Bos1*. Like *Bos1*, *Sec22* is an abundant component of endoplasmic reticulum to Golgi transport vesicles⁵.

Bos1, *Snc1* and *Snc2* are thus key elements in the targeting and fusion of vesicles. For their neuronal counterpart, synaptobrevin, comparable genetic evidence is unavailable, and the first clue to its function came instead from the discovery that it is the target of certain neurotoxins produced by bacteria of the genus *Clostridium*^{6,7}. Clostridial neurotoxins have long been known to be responsible for the clinical manifestations of tetanus and botulism. Seven distinct botulinum neurotoxins (designated BoNT/A, B, C1, D, E, F and G) and one tetanus toxin are known (reviewed in refs 31, 32). All are homologous heterodimers. The heavy chains mediate cell entry by binding selectively to certain neurons. Once internalized, the light chains block synaptic vesicle exocytosis by acting as Zn^{2+} -dependent proteases specific for the membrane-bound constituents of the neuronal fusion complex. Synaptobrevin, the first substrate identified, is cleaved by most of the toxins (tetanus toxin, botulinum neurotoxins type B, D and F^{6,7,10,33}), implicating it in exocytotic membrane fusion and indicating that it is the neuronal counterpart of yeast *Snc1/Snc2*.

SNAP-25

Despite their structural similarity, the substrates of clostridial neurotoxins are mutually exclusive. None of the toxins that cleave synaptobrevin affect any other identified proteins and three of the botulinum toxins (type A, C1 and E) that do not interact with synaptobrevin cleave either syntaxin (type C1⁹) or SNAP-25 (types A and E^{8,10,33}). The finding that SNAP-25 is part of the fusion complex was a surprise as this protein was until recently thought to be involved in neurite formation³⁴. It may be anchored to membranes by palmitoyl side chains attached to cysteine residues³⁵. Toxin cleavage occurs near the C terminus, releasing a small peptide⁸. SNAP-25 is 19% identical to

TABLE 1 Yeast gene products structurally related to neuronal components

Gene product	Stage of transport	Relative
<i>BOS1</i> *	ER-Golgi	Synaptobrevin
<i>SEC22</i>	ER-Golgi	Synaptobrevin
<i>BET1</i>	ER-Golgi	Synaptobrevin
<i>SNC1</i> *	Post-Golgi	Synaptobrevin
<i>SNC2</i> *	Post-Golgi	Synaptobrevin
<i>SED5</i> *	ER-Golgi	Syntaxin
<i>PEP12</i>	Golgi-vacuole	Syntaxin
<i>SSO1</i> *	Post-Golgi	Syntaxin
<i>SSO2</i> *	Post-Golgi	Syntaxin
<i>SEC9</i>	Post-Golgi	SNAP-25

Proteins that are similar in structure to synaptobrevin, syntaxin and SNAP-25 have been identified in *Saccharomyces cerevisiae*. Some of these gene products are significantly homologous to their neuronal counterparts. Genes whose products share >25% identity to each of their relatives are marked by an asterisk. ER, endoplasmic reticulum.

the C-terminal domain (231 amino acids) of Sec9 (V. Bankaitis, personal communication). This part of Sec9 is required for the fusion of post-Golgi transport vesicles with the plasma membrane in yeast (ref. 23; V. Bankaitis, personal communication).

Syntaxin

Both isoforms of syntaxin, the third component of the neuronal complex, are anchored to membranes by a single C-terminal transmembrane domain^{36,37}. Toxin cleavage of syntaxin inhibits neurotransmitter release⁹, indicating that syntaxin is directly involved in exocytotic membrane fusion. Such a role is also supported by the observation that microinjection of syntaxin fragments or anti-syntaxin antibodies reduces exocytosis in PC12 cells²⁷.

As well as forming a complex with SNAP-25 and synaptobrevin¹¹, syntaxin also binds to munc-18 (also called rbSec1, see below), to presynaptic Ca²⁺ channels and to the Ca²⁺-binding protein synaptotagmin located on synaptic vesicles^{36,38,39}. Genetic studies and microinjection experiments indicate that synaptotagmin may be an important regulator of neuronal exocytosis⁴⁰⁻⁴⁵. *In vitro*, synaptotagmin interacts with the syntaxin/synaptobrevin/SNAP-25 complex, apparently in competition with α -SNAP¹⁸, but whether it acts as the major exocytotic Ca²⁺ regulator that releases the fusion complex on Ca²⁺ binding remains unclear.

Proteins related to the syntaxins that have also been identified on yeast target membranes include the products of the *SSO1*, *SSO2*, *SED5* and *PEP12* genes. Each of these proteins functions at different stages of the yeast secretory pathway^{4,44,45}. As is the case for the synaptobrevin-like proteins, those proteins that act late in the secretory pathway (*Sso1* and *Sso2*) are most closely related to the neuronal syntaxins, perhaps because neurotransmitter release is a post-Golgi transport event.

The products of *SSO1* and *SSO2*, which were identified as high-copy suppressors of *sec1*, are closely related to the syntaxins and are 72% identical to each other. Neither is essential for the growth of yeast cells but disruption of both genes leads to a block in post-Golgi transport and is lethal⁴. *Sso1* and *Sso2* are also related to *Sed5* and to *Pep12*, a protein required for vacuolar sorting^{4,45}. *Sed5* is required for endoplasmic reticulum to Golgi transport and the bulk of this protein is located in a punctate structure which is probably the Golgi apparatus⁴⁴. *Sed5* may therefore be the Golgi complex t-SNARE that interacts with *Bos1* (and possibly *Sec22*), whereas *Sso1/Sso2* may interact with *Snc1/Snc2* (see Fig. 1).

Rabs

Members of a family of small GTP-binding proteins, called Rabs in mammalian cells and *Ypt* and *Sec4* in yeast, have also been implicated in intracellular membrane fusion. The Rabs were once proposed to be molecular 'tags' directing the transport vesicle to its correct target (reviewed in ref. 46), but recent findings indicate that they in fact act as 'switches' regulating vesicle fusion^{47,48}. A single chimaera of the yeast proteins *Ypt1* and *Sec4*, which function, respectively, in endoplasmic reticulum to Golgi and post-Golgi transport, can function at both stages of the pathway without detectable missorting. Rather than being involved in membrane recognition, the Rabs may therefore regulate the assembly of docking/fusion complexes at different transport steps.

The search for genes that can compensate for the loss of *YPT1* led to the isolation of *SLY1*, as well as the re-isolation of *BET1* and *SEC22* (ref. 49). *Sly1* is required for endoplasmic reticulum to Golgi transport⁵⁰, and belongs to a new family of soluble proteins which appear to be essential for intracellular fusion events. Other members of this family include *Sec1* and *Slp1*⁵¹ (also called *Vps33*; ref. 52) which function in exocytosis and Golgi to vacuolar transport, respectively. The ability of overexpressed *Sso1* and *Sso2* to suppress mutations in *SEC1* suggests that these proteins interact with the *Sec1* protein. Similarly, *Sly1* may bind to *Sed5*, the syntaxin homologue that acts in endo-

plasmic reticulum to Golgi transport. Syntaxin indeed binds to a mammalian homologue of *Sec1* and to the product of the *C. elegans UNC-18* gene, munc-18 or rbSec1 (refs 53, 54). The binding of munc-18 requires the N terminus of syntaxin, whereas the C terminus is needed for interaction with SNAP-25⁵³, indicating that syntaxin-like proteins may engage in multiple protein-protein interactions using distinct domains.

The past few years have given us our first glimpse of the apparatus responsible for eukaryotic membrane fusion (as shown in Fig. 1 and reviewed in refs 55, 56). Most if not all eukaryotic docking/fusion machines may thus be structurally similar, offering a multiplicity of experimental systems to address specific questions raising a new and fascinating set of problems. For example, are the SNAREs involved in docking, fusion, or both steps? Are the known components of this docking/fusion complex sufficient to carry out membrane fusion, or are additional constituents required? Even if they are sufficient, other proteins, some of them tissue-specific, may modulate their activity and GTP-binding proteins may control the activity of any part of the fusion complex. The answers to these questions will maintain the excitement in the field for some time to come. □

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A dwarf satellite galaxy in Sagittarius

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WE have detected a large, extended group of comoving stars in the direction of the Galactic Centre, which we interpret as belonging to a dwarf galaxy that is closer to our own Galaxy than any other yet known. Located in the constellation of Sagittarius, and on the far side of the Galactic Centre, it has not previously been seen because of the large number of foreground stars (in the Milky Way) in that direction. Following convention, we propose to call it the Sagittarius dwarf galaxy. Its properties are similar to those of the eight other dwarf spheroidal companions to the Milky Way, and it is comparable in size and luminosity to the largest of them—the Fornax system. The Sagittarius dwarf is elongated towards the plane of the Milky Way, suggesting that it is undergoing some tidal disruption before being absorbed by the Milky Way.

As part of an investigation into stellar abundances and kinematics in the outer regions of the Galactic bulge (R.A.I. and G.G., manuscript in preparation) 18 regions were initially selected for study from the reddening maps of Burstein and Heiles¹, along lines of sight known to have low extinction. Candidate K and M giant stars were chosen for spectroscopic follow-up on the basis of automatic plate measuring (APM) scans of UK Schmidt Telescope (UKST) B_J and R plates. The spectroscopic data were collected over three observing runs using the 3.9-m Anglo-Australian Telescope (AAT) equipped with the multi-fibre system AUTOFIB. This spectrograph allows simultaneous study of some of some 64 objects, of which 50 are typically targets of direct interest, with the remaining fibres being used for guiding and sky estimation. A total of 40 fibre fields chosen from 6 of the 18 selected regions were observed in this way, and reduced in the standard manner. Radial velocities for all the target stars, accurate to 9 km s^{-1} , were then derived by cross-correlating each target spectrum with radial velocity standard star templates².

Figure 1 shows the derived heliocentric radial velocity as a function of $(B_J - R)$ colour for three selected regions at Galactic coordinates longitude $l = 5^\circ$, latitude $b = -12^\circ, -15^\circ, -20^\circ$. The broad distribution of stars with mean near 0 km s^{-1} and with dispersion $\sim 70 \text{ km s}^{-1}$ is the expected bulge population. However, the low velocity dispersion group of stars at a common velocity of $\sim 140 \text{ km s}^{-1}$ extending to $(B_J - R)$ colours > 3 , is not predicted by any Galaxy model. This feature does not appear in any of the other regions for which spectroscopy has been obtained. The excess of stars is most prominent at $l = 5^\circ, b = -15^\circ$ but is also unambiguously present in the other two regions implying a spatial extent of at least 8° .

After accounting for the expected velocity distribution, we fit the velocity of the unexpected population with a gaussian distribution, and find that the systemic heliocentric radial velocity of the comoving stars is $+140 \pm 2 \text{ km s}^{-1}$ (172 km s^{-1} in a Galactocentric system) with a velocity dispersion, corrected for measuring errors, of $\sim 10 \text{ km s}^{-1}$. There is no significant radial velocity difference, $< 5 \text{ km s}^{-1}$, between the stars at $b = -12^\circ$ and -20° . The velocity dispersion is typical of Galactic satellite systems, but is a factor of ~ 6 smaller than the dispersion observed in Galactic bulge stars in the same fields (R.A.I. and G.G., manuscript in preparation) and ~ 10 times lower than the dispersion observed in Galactic disk stars at similar longitudes³.

In order to further investigate this feature, the APM facility⁴ was used to measure $5.3^\circ \times 5.3^\circ$ areas of the four UKST survey

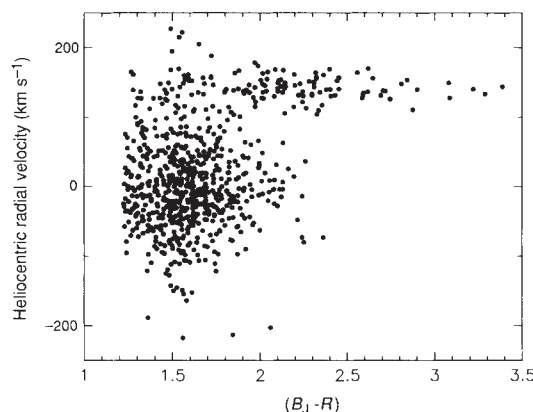
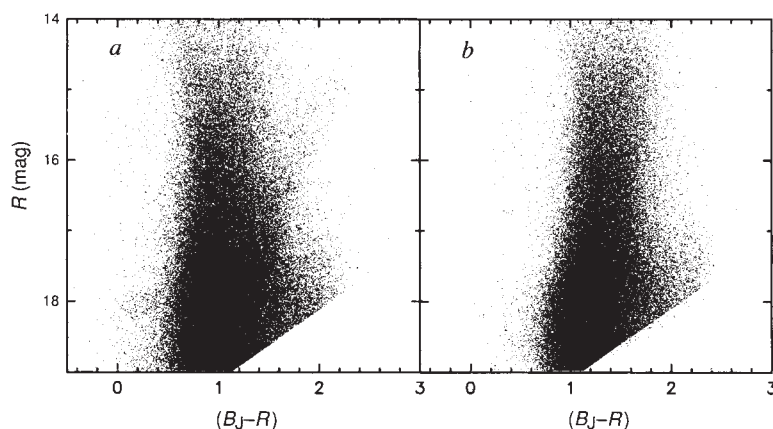


FIG. 1 The heliocentric radial velocity–colour distribution for three selected regions at Galactic coordinates $l = 5^\circ, b = -12^\circ, -15^\circ$ and -20° . The population with mean velocity of 0 km s^{-1} and dispersion 70 km s^{-1} is the expected Galactic bulge. There is a clear excess of stars at a common velocity of $\sim 140 \text{ km s}^{-1}$ extending to $(B_J - R)$ colours > 3 . This feature does not appear in any of the other regions for which spectroscopy has been obtained. The excess of stars is most prominent at $l = 5^\circ, b = -15^\circ$ but is also unambiguously present in the other two regions.

fields that straddle this region of sky. A deep $2^\circ \times 2^\circ$ CCD (charge-coupled device)-calibrated photographic colour–magnitude diagram (CMD) centred at right ascension (RA) 18 h 51.9 min, declination (dec.) $-30^\circ 33'$ (B1950) (that is, $l = 5.5^\circ, b = -14.1^\circ$) clearly reveals (Fig. 2a) the following: a red horizontal branch/red giant clump at $R = 17.6 \text{ mag}$, $(B_J - R) = 1.3$ with traces of a long extension towards the blue to $(B_J - R) = 0$; a steeply rising red giant branch extending to $R = 14 \text{ mag}$, $(B_J - R) = 3.0$; and a large Galactic foreground population occupying the rest of the domain. From the maps of Burstein and Heiles¹ we estimate the extinction in $(B - V)$ to be 0.14, which is equivalent to a total extinction in the R band of 0.32 mag. An alternative estimate of the reddening can be obtained by comparing the horizontal and giant branch morphology with a similar photographic CMD showing the intermediate age population of the Small Magellanic Cloud (SMC)⁵. By translating the SMC diagram 1.6 mag brighter in R and 0.2 mag towards the red in $(B_J - R)$ an excellent match ensues. The reddening estimated this way amounts to ~ 0.3 mag of extinction in R , in good agreement with the previous value. Adopting this directly determined reddening, and assuming a distance to the SMC of 57 kpc (ref. 6) leads to a preliminary distance estimate of $24 \pm 2 \text{ kpc}$ for the Sagittarius dwarf. The good match with an SMC intermediate age stellar population CMD suggests that a significant intermediate age population is present in the Sagittarius dwarf; the weak extension towards the blue of the horizontal branch would imply that there is also an underlying old stellar population present. This also is typical of Galactic dwarf spheroidal satellites and implies that a significant population II variable star component should be present.

Figure 2b shows a comparison CMD, constructed from photometry from the same photographic plates as Fig. 2a, but centred on $l = 8.4, b = -13.5$. Though the reddening in this region is almost identical to that at $l = 5.5^\circ, b = -14.1^\circ$, only features typical of the Galactic bulge and disk are visible. We have generated $2^\circ \times 2^\circ$ CMDs down to $R = 19 \text{ mag}$ in several locations on the four UKST fields (396, 397, 458 and 459) that lie near $l = 5^\circ, b = -15^\circ$, and on UKST fields 336 and 517 which lie on the other side of the bulge minor axis at $l = -5^\circ, b = -15^\circ$, and $l = -5^\circ, b = 15^\circ$ respectively. We have also obtained $1^\circ \times 1^\circ$ CMDs down to $R = 17 \text{ mag}$ at the positions of the 18 selected regions which lie near the intersections of $l = 25^\circ, 15^\circ, 5^\circ, -5^\circ, -15^\circ$ and -25° , and $b = -12^\circ, -15^\circ$ and -20° . The distinctive giant branch and horizontal clump are only visible in UKST fields 458 and 459.

FIG. 2 A photographic colour-magnitude diagram covering $2^\circ \times 2^\circ$ of sky centred at RA 18 h 51.9 min, dec. $-30^\circ 33'$ (B1950). Note the red horizontal branch/red giant clump at $R=17.6$ mag, $(B_J - R)=1.3$, the extension towards the blue to $(B_J - R)=0$ and a steeply rising red giant branch extending to $R=14$ mag, $(B_J - R)=3.0$. The rest of the diagram is dominated by Galactic foreground stars. *b*, A comparison colour-magnitude diagram constructed from photometry from the same photographic plates as *a*, but centred on $l=8.4^\circ$, $b=-13.5^\circ$. Though the reddening in this region is almost identical to that at $l=-5.5^\circ$, $b=-14.1^\circ$, only features typical of the Galactic bulge and disk are visible.



The presence of a well populated horizontal branch provides a convenient way to determine the spatial extent and luminosity of the galaxy against the contamination from the Galactic foreground stars. The large gradient due to Galactic foreground stars precludes direct inspection of the spatial extent of the dwarf galaxy. A simple solution is to produce an isopleth (isodensity) map containing images selected to be predominantly at the horizontal branch magnitude, and subtract off from it an equivalent isopleth map containing images at different magnitudes. The overdensity of the dwarf at the horizontal branch magnitude then reveals the underlying dwarf galaxy. Our preliminary mapping of four UKST fields 396, 397, 458 and 459 in this way, shows that the Sagittarius dwarf extends over fields 458 and 459 but is not apparent in the adjacent fields. Figure 3 shows the distribution of horizontal branch stars obtained in fields 458 and 459. The dwarf galaxy is elongated along a direction pointing roughly towards the Galactic Centre, the elongation and general morphological appearance is indicative of continuing tidal disruption. There are four catalogued globular clusters in the vicinity of the dwarf galaxy (M54, Arp2, Terzan7 and Terzan8); they may be associated with the Sagittarius system, as they lie at a similar distance and have similar velocities. M54, at RA 18 h 51.9 min, dec. $-30^\circ 32'$, lies at the centre of the densest clump of stars in the Sagittarius dwarf, although due to the central 4 arcmin being completely saturated on the B_J survey plate it contributes little to either the overall star counts or the CMD of Fig. 2*a*. Arp2, Terzan7 and Terzan8 lie along the eastern and southern periphery outside the region shown in Fig. 3. With the present published uncertainties in the distance and radial velocities of these clusters we are unable to confirm if any of them are members of the Sagittarius dwarf.

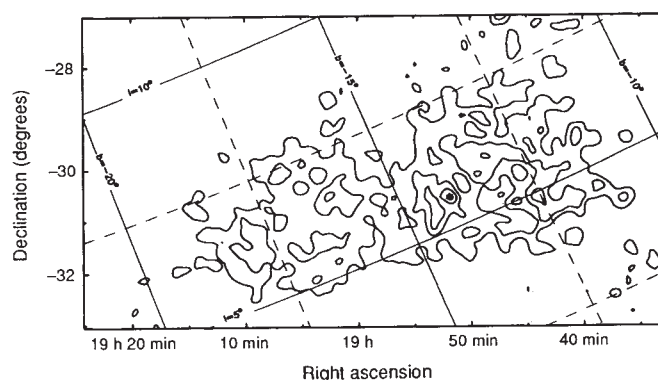
We have estimated the metallicity of the Sagittarius dwarf by converting the (B_J, R) magnitudes of Fig. 2*a* into (B, V) , and then comparing the colour of the giant branch with other Galactic dwarf spheroidal galaxies (dSphs) and globular clusters⁷. The Fornax dSph provides the closest match suggesting that the mean metallicity $[\text{Fe}/\text{H}] \approx -1.4 \pm 0.3$. This is consistent with

$[\text{Fe}/\text{H}] \approx -1 \pm 0.3$, estimated spectroscopically (using the equivalent width of the $\text{Mg } \text{I } 'b'$ feature) from candidate member stars. The spread seen in the spectroscopically derived metallicity distribution is similar to the metallicity spread (0.3dex) of Fornax⁷. If the dwarf galaxy follows the general trend of metallicity versus absolute luminosity⁸, it should be among the brightest Galactic dSphs. The luminosity of such an extended system is difficult to estimate directly but by comparing the number of horizontal and giant branch stars with the other Galactic dSphs it is possible to make a provisional estimate. The integral number of horizontal branch stars shown in the isopleth map of Fig. 3 is $\sim 17,000$. For low density dSphs the outermost traceable isophote typically represents $\sim 50\%$ of the whole population⁹. The Fornax dSph contains a total of $\sim 35,000$ horizontal branch stars¹⁰. With the caveat that the evolutionary history and hence detailed stellar mix may be different, the number of stars in Sagittarius and Fornax is therefore similar, implying an absolute luminosity for Sagittarius of $M_V \approx -13$ mag.

Four of the stars with velocities $\sim 140 \text{ km s}^{-1}$ show spectroscopic features typical of cool carbon stars; several more carbon star candidates have been found in this region from visual inspection of UKST objective prism plates (D. Morgan, personal communication). Their appearance implies the presence of a significant intermediate age component, which is consistent with the findings from the CMD. The only other Galactic dSph with a large carbon star population is Fornax.

Is this Sagittarius dwarf galaxy a dwarf irregular or dSph galaxy? We have searched currently available H I maps (21-cm emission) of the region and find no evidence of H I emission associated with the dwarf. However, none of the existing maps have the necessary velocity and spatial resolution to completely rule out the presence of H I . We have also thoroughly searched the photographic plates for evidence of any associations of young blue stars that would favour the dwarf irregular interpretation. The only excess of blue stars appears at the level of the horizontal branch. Our preliminary interpretation of the satellite

FIG. 3 Isopleth maps for UKST fields 458 and 459 constructed from the excess of images present at the horizontal branch magnitude. The lowest contour is $1/8 \text{ image arcmin}^{-2}$. Contours increment by $1/2 \text{ image arcmin}^{-2}$. The dwarf galaxy has been disrupted into two distinct clumps of stars. The Sagittarius dwarf has a spatial extent of 3 kpc and an absolute visual magnitude of $M_V = -13$, assuming a distance of 24 kpc from the Sun. We estimate the Galactocentric distance to be 16 kpc.



as a dSph is not yet conclusive, but the similarities between the dwarf and the rest of the Galactic dSphs are compelling.

The close proximity to the Galactic Centre, the morphological appearance and the radial velocity of 140 km s^{-1} indicate that this system is currently undergoing strong tidal disruption before being integrated into the Milky Way. The tidal limit for a bound system of similar dynamical mass to Fornax, at a distance of 16 kpc from the Galactic Centre, is ~ 0.5 kpc. As Sagittarius extends over at least 3 kpc, almost an order of magnitude larger than the tidal limit, it provides us with a snapshot of an early phase of the tidal destruction of a satellite galaxy. $\square$

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Preparation and structure of crystals of the metallofullerene $\text{Sc}_2@\text{C}_{84}$

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It was first proposed in 1985¹ that fullerenes can confine atoms in their interior because of their closed-cage structure. Attempts to verify this conjecture following the mass production of fullerenes^{2,3} have yielded metallofullerenes in bulk, and there is now good evidence that these compounds are endohedral⁴—that is, that the metal atoms are inside. But direct confirmation in the form of structural data has been lacking, in part because of the difficulty of separating different metallofullerenes and obtaining pure crystals. Here we report the preparation of pure crystalline $\text{Sc}_2@\text{C}_{84}$ and analyses of its structure by electron diffraction and high-resolution transmission electron microscopy. At room temperature the $\text{Sc}_2@\text{C}_{84}$ molecules pack in a hexagonal-close-packed structure with a ratio of lattice constants $c/a = 1.63$, the value expected for ideal-sphere packing. The molecular spacing of 11.2 Å is the same as that found earlier in crystalline C_{84} (refs 5, 6). The match between our microscopic images and simulations is markedly better for endohedral models than for those in which the metal atoms reside in the lattice outside the C_{84} cages. We believe that this combination of observations points inevitably to the conclusion that the metal atoms are inside the fullerenes.

Interest in metallofullerene compounds is motivated by the possibility that appropriate internal dopants can be used to tune the physical and chemical properties of the molecules, giving rise

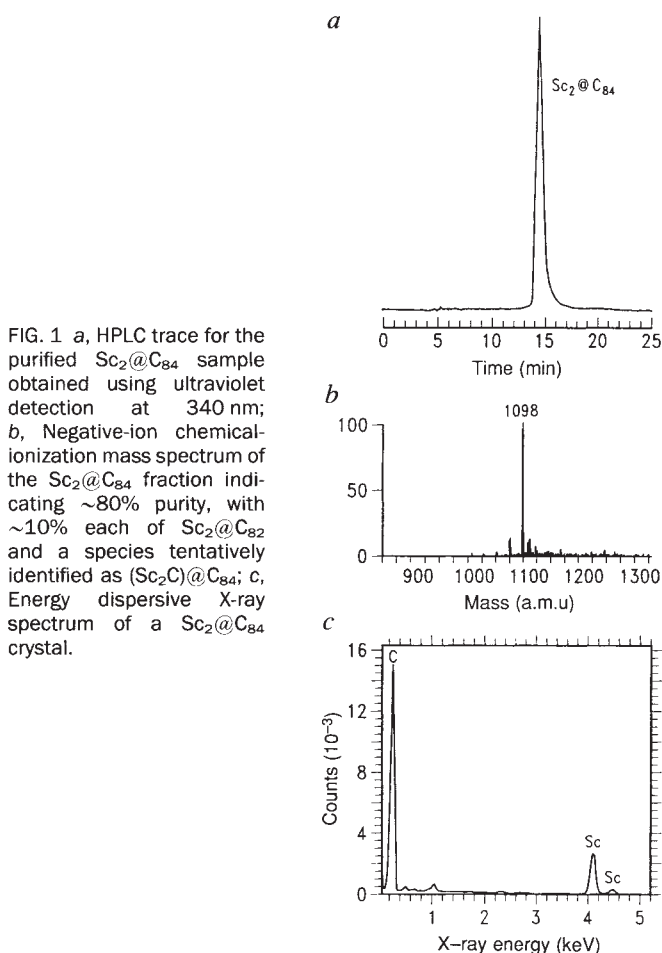


FIG. 1 a, HPLC trace for the purified $\text{Sc}_2@\text{C}_{84}$ sample obtained using ultraviolet detection at 340 nm; b, Negative-ion chemical ionization mass spectrum of the $\text{Sc}_2@\text{C}_{84}$ fraction indicating $\sim 80\%$ purity, with $\sim 10\%$ each of $\text{Sc}_2@\text{C}_{82}$ and a species tentatively identified as $(\text{Sc}_2\text{C})@\text{C}_{84}$; c, Energy dispersive X-ray spectrum of a $\text{Sc}_2@\text{C}_{84}$ crystal.

to a family of materials with potential applications as catalysts, nonlinear optical or ferroelectric materials, transport agents for refractory or reactive species, photosensitizers or electronic materials. Metallofullerenes containing Y, Sc or lanthanide atoms form readily, and have been characterized by a variety of methods⁴, but detailed characterization and structural studies have been impeded by the difficulty of preparing pure samples. Methods for purifying these species using multiple stages of high-performance liquid chromatography (HPLC) have been reported recently^{7–10}, and initial characterization work, including ultraviolet-visible spectroscopy^{7,8}, infrared spectroscopy⁸ and scanning tunnelling microscopy¹¹ has been done.

We prepared metallofullerenes by arc-vaporizing 6-mm diameter cored-carbon rods, packed with a mixture of Sc or Sc_2O_3 and graphite powders, in 200 torr of helium with ~ 100 A direct current. Metal loadings of 1–3 at% (with respect to total carbon) were used. The soot was promptly extracted with CS_2 . Purification was accomplished by methods similar to those described in the literature^{7,8}, but with the HPLC system fully automated to allow unattended operation⁹, and equipped with both ultraviolet and on-line electron paramagnetic resonance (EPR) detection to allow isolation of fractions containing mono-metal and trimetal metallofullerenes¹⁰. A toluene/decalin (80/20) mixture with relatively high solubility for fullerenes and metallofullerenes was used as the eluent, allowing relatively large quantities of material (≥ 10 mg) to be injected onto the columns. Coarse separation of metallofullerenes from empty fullerenes was achieved using polystyrene columns. A second HPLC stage using a Buckyclutcher (Regis Chemical Co., Morton Grove, Illinois) column separated the different metallofullerenes. Further details will be published elsewhere^{9,10}. The dominant scandium metallofullerenes were discandium species with cages of 74, 76,

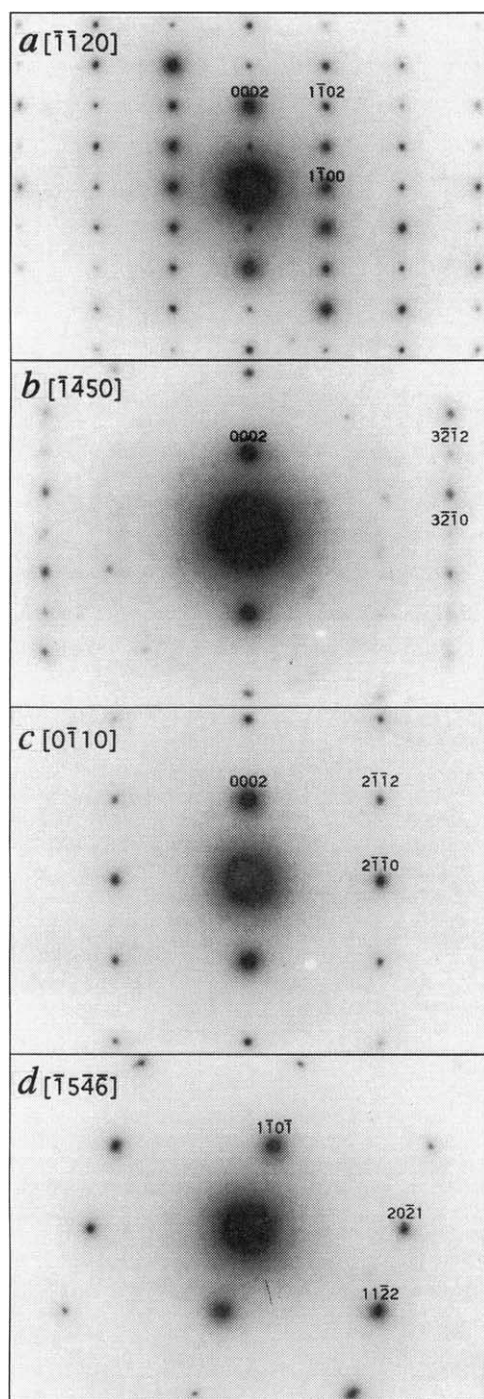


FIG. 2 Electron diffraction zone-axis patterns of $\text{Sc}_2\text{@C}_{84}$, taken along the $[1120]$ (a), $[1450]$ (b), $[0110]$ (c) and $[1546]$ (d) directions.

or even numbers of carbons between 80–104, with $\text{Sc}_2\text{@C}_{84}$ the most abundant^{9,10}. Figure 1 shows analytical data for the purified $\text{Sc}_2\text{@C}_{84}$. An HPLC trace for the final $\text{Sc}_2\text{@C}_{84}$ sample is shown in Fig. 1a, and the corresponding negative-ion chemical-ionization mass spectrum is shown in Fig. 1b. $\text{Sc}_2\text{@C}_{84}$ constitutes ~80% of this sample, with $\text{Sc}_2\text{@C}_{82}$ accounting for an additional 10%. A peak near 1,110 atomic mass units (a.m.u.) (also ~10%), may be due to $(\text{Sc}_2\text{C})\text{@C}_{84}$, $\text{Sc}_2\text{@C}_{84}\text{O}$ and/or $\text{ScO}_2\text{C}_{86}$. A number of peaks possibly corresponding to odd-carbon metallofullerenes were found in other HPLC fractions. A second $\text{Sc}_2\text{@C}_{84}$ isomer (confirmed by mass spectrometry) was completely resolved by HPLC at a retention time shorter

than that for the major $\text{Sc}_2\text{@C}_{84}$ isomer studied here. This second species was also obtained as a pure sample.

Crystals were grown by dissolving the $\text{Sc}_2\text{@C}_{84}$ in CS_2 and allowing the solvent to evaporate slowly. These crystals were suspended in ethanol and placed on a holey carbon grid for examination by transmission electron microscopy (TEM), performed in a Topcon 002B instrument operating at either 100 or 200 kV. The lower voltage was used for high-resolution imaging because electron-beam damage occurred quite rapidly when a 200 kV beam was focused onto a $\text{Sc}_2\text{@C}_{84}$ crystal. Crystals with dimensions ranging from 1 to 20 μm were observed. Elemental analysis of the crystals by energy dispersive X-ray spectroscopy (EDS) showed principally carbon and scandium (Fig. 1c). Sulphur (presumably from CS_2) could also be detected in some crystals, but was absent from others ($\text{S}/\text{Sc} < 0.05$).

Electron diffraction patterns from sulphur-free crystals are shown in Fig. 2. These patterns indicate that $\text{Sc}_2\text{@C}_{84}$ crystallizes in a hexagonal-close-packed (h.c.p.) structure, with lattice parameters $a = 11.2 \pm 0.2 \text{ \AA}$ and $c = 18.3 \pm 0.2 \text{ \AA}$, giving a c/a ratio of 1.63. The observed reflections are consistent with the hexagonal space group $P6_3/mmc$. In Fig. 2a, the (0001) and (0003) reflections arise because of double diffraction; these reflections disappeared when the crystal was tilted slightly off the $[1120]$ zone axis. No streaks were observed in the diffraction patterns, indicating that no stacking faults were present that would locally change the h.c.p. structure to a face-centred cubic (f.c.c.) structure.

High-resolution TEM images looking along the orthogonal $[0001]$ and $[1120]$ directions are shown in Figs 3 and 4, respectively. In an h.c.p. structure, the close-packed planes have an ABAB... stacking sequence along the c axis, whereas the close-packed planes in an f.c.c. structure have an ABCABC... stacking sequence. The ABAB... stacking expected for an h.c.p. structure is evident in Fig. 4, and in Fig. 3 the prominent bright dots correspond to the aligned columns of unoccupied C sites. These images also confirm directly the absence of stacking faults and the lattice parameters measured by electron diffraction.

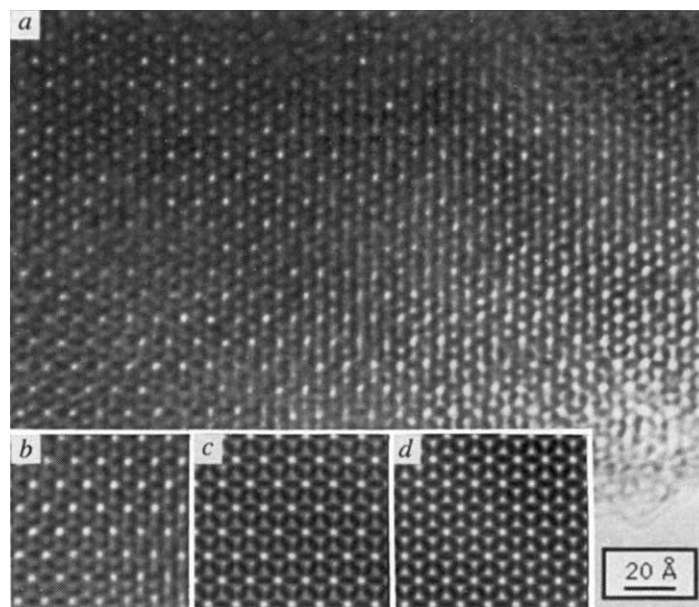
Detailed consideration of high-resolution TEM images such as those in Figs 3 and 4, and the electron diffraction data in Fig. 2, leads us to conclude that the scandium atoms in $\text{Sc}_2\text{@C}_{84}$ are inside the carbon cage. In thin ($\leq 100 \text{ \AA}$) fullerene crystals near Scherzer defocus, there is a one-to-one correspondence between the projected crystal potential and the high-resolution image¹². For example, columns of empty C_{60} and C_{70} are imaged as dark rings around a bright central spot¹³.

The TEM image in Fig. 3a looks down the c axis of a $\text{Sc}_2\text{@C}_{84}$ crystal. The black features correspond to the overlapping projections of the carbon cages of molecules centred at the A and B sites, whereas the grey features in Fig. 3a–c are centred on these molecules. Comparing these grey features with the corresponding (but brighter) areas in the simulated image for empty C_{84} (Fig. 3d) shows that there is additional projected charge density within columns of $\text{Sc}_2\text{@C}_{84}$ molecules for this viewing direction.

The TEM image in Fig. 4a was obtained with the crystal oriented so that the $\text{Sc}_2\text{@C}_{84}$ molecules are stacked in columns normal to the image plane. The columns of molecules appear as dark circles, indicating that the additional charge density associated with the Sc atoms is located within these columns. This result is supported by comparing the image with simulated TEM images for empty (Fig. 4d) and Sc-containing (Fig. 4c) C_{84} molecules arranged in an h.c.p. structure. The simulation for the empty cages shows the expected bright central spots, which are absent from the image for the metal-filled species. Taken together, these projected images looking along two perpendicular crystal directions provide strong evidence that the metal atoms are within the C_{84} cages. This location is further supported by the diffraction data, which shows that the nearest-neighbour separation for $\text{Sc}_2\text{@C}_{84}$ is the same as that found previously for C_{84} ($11.2 \text{ \AA}$)^{5,6}.

NMR studies^{14,17} and theoretical calculations^{18,22} indicate

FIG. 3 *a*, High-resolution TEM image of a $\text{Sc}_2@\text{C}_{84}$ crystal, taken along the [0001] direction. Inset *b* is a Fourier-filtered image that brings out the periodicities present in the original image. Insets *c* and *d* are simulated images of 67 Å thick $\text{Sc}_2@\text{C}_{84}$ and C_{84} crystals, respectively, at -500 Å defocus. In *c* and *d*, three different orientations of the molecules are superimposed to simulate the orientational disorder present in the crystals.



that empty C_{84} consists of a 2:1 mixture of $D_{2d}(22)$ and $D_{2d}(23)$ isomers (in the notation of Manolopolous and Fowler²³). The presence of analogous isomers of $\text{Sc}_2@\text{C}_{84}$ would result in disorder within the crystals. Moreover, the fact that $\text{Sc}_2@\text{C}_{84}$ crystals have the c/a ratio expected for hexagonal close-packing of perfect spheres ($c/a = \sqrt{8/3} \approx 1.63$), even though the isomers of the $\text{Sc}_2@\text{C}_{84}$ molecule are not perfectly spherical, indicates that there is orientational disorder (either static or dynamic) of the molecules within the crystals at room temperature. This conclusion is supported by comparison of experimental and simulated high-resolution images. The match between these images is improved if several different orientations of the $\text{Sc}_2@\text{C}_{84}$ molecule are superimposed (Figs 3 and 4), rather than having unique, fixed positions for the Sc and C within the unit cell.

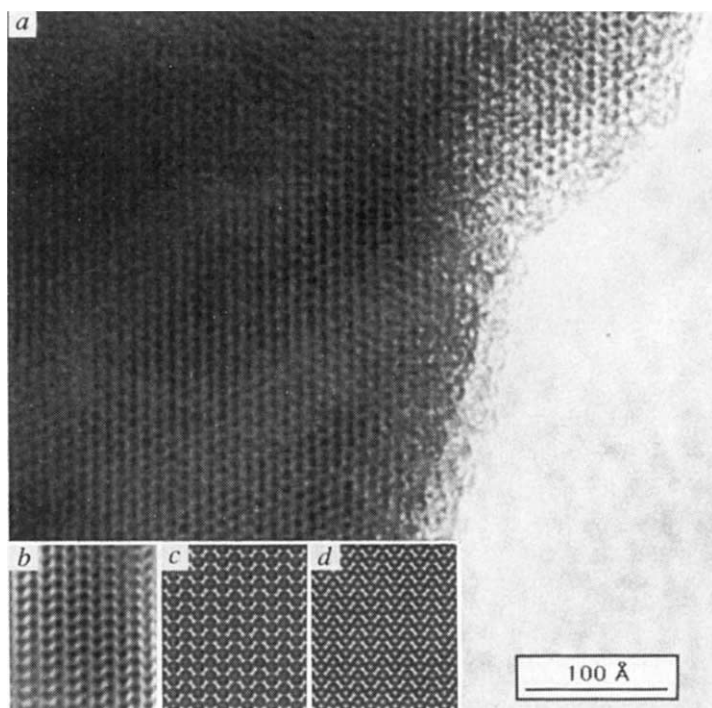
The matching intermolecular spacing in $\text{Sc}_2@\text{C}_{84}$ and C_{84} indicates that possible charge transfer to the cage in $\text{Sc}_2@\text{C}_{84}$ does

not significantly increase its van der Waals radius. The C_{84} cage diameter (extrapolated from 7.1 Å for C_{60} by assuming equal surface densities of carbon atoms) is estimated to be 8.4 Å. Thus the distance between the carbon cages in crystals of both C_{84} and $\text{Sc}_2@\text{C}_{84}$ is ~ 2.8 Å. This can be compared with the cage spacing of 2.87 Å for C_{60} crystals derived from the ball diameter²⁴ and lattice parameter²⁵ found in earlier studies. The conclusion that the van der Waals radii of $\text{Sc}_2@\text{C}_{84}$ and C_{84} are the same contrasts with the conclusion drawn from STM studies¹¹ that $\text{Sc}@\text{C}_{74}$ and $\text{Sc}_2@\text{C}_{74}$ have larger diameters than would be expected from the diameter of the corresponding empty fullerene cage (~ 7.9 Å), possibly due to charge transfer from the metal atoms to the cage. □

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FIG. 4 *a*, High-resolution TEM image of a $\text{Sc}_2@\text{C}_{84}$ crystal, taken along the [1120] direction. Inset *b* is a Fourier-filtered image that brings out the periodicities present in the original image. Insets *c* and *d* are simulated images of 22 Å thick $\text{Sc}_2@\text{C}_{84}$ and C_{84} crystals, respectively, at -500 Å defocus. In *c* and *d*, orientational disorder is simulated by the same method employed in Fig. 3.



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Biological magnetic resonance imaging using laser-polarized ^{129}Xe

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As currently implemented, magnetic resonance imaging (MRI) relies on the protons of water molecules in tissue to provide the NMR signal. Protons are, however, notoriously difficult to image in some biological environments of interest, notably the lungs¹ and lipid bilayer membranes such as those in the brain². Here we show that ^{129}Xe gas can be used for high-resolution MRI when the nuclear-spin polarization of the atoms is increased by laser optical pumping and spin exchange^{3–6}. This process produces hyperpolarized ^{129}Xe , in which the magnetization is enhanced by a factor of about 10^5 . By introducing hyperpolarized ^{129}Xe into mouse lungs we have obtained images of the lung gas space with a speed and a resolution better than those available from proton MRI^{1,7} or emission tomography^{8,9}. As xenon (a safe general anaesthetic) is rapidly and safely transferred from the lungs to blood and thence to other tissues^{8,9}, where it is concentrated in lipid^{10–15} and protein^{13,15–18} components, images of the circulatory system, the brain and other vital organs can also be obtained. Because the magnetic behaviour of ^{129}Xe is very sensitive to its environment, and is different from that of $^1\text{H}_2\text{O}$, MRI using hyperpolarized ^{129}Xe should involve distinct and sensitive mechanisms for tissue contrast.

The magnetic resonance signal strength of a given nuclear species depends on its total magnetization in the chosen sample volume element; that is, on the product of the species concentration, the excess spin density per nucleus (polarization), and the volume of the element. In the largest available magnetic fields, thermal-equilibrium polarizations reach $\sim 10^{-5}$, which is

adequate for $^1\text{H}_2\text{O}$ imaging. Other proton species, and all other nuclear species, have concentrations too small to be of use in conventional high-speed, high-resolution MRI. Xenon gas at 1 atm, for example, has a molar concentration only 0.04% that of tissue $^1\text{H}_2\text{O}$. However, the polarization of ^{129}Xe and other spin 1/2 noble gases can be enormously enhanced through spin-exchange with an optically pumped alkali metal vapour^{3–5}. In our case, a dilute vapour of rubidium (~ 1 p.p.m. of the Xe pressure) is kept spin-polarized by the absorption of 1–2 W of circularly polarized 795-nm Rb D_1 -resonance light from a Ti:sapphire laser. Gas-phase collisions between the ^{129}Xe and the polarized Rb atoms result in the transfer of angular momentum from the Rb valence electron to the ^{129}Xe nucleus. The ^{129}Xe gas is laser-polarized to at least 25% in 5–20 min (depending on conditions), enhancing its NMR signal to $\sim 10^5$ times the thermal equilibrium value. The gas is then delivered for imaging.

Figure 1a–c shows the evolution in time (0–10 s) of the distribution of hyperpolarized ^{129}Xe entering the lungs of a mouse lung-heart preparation. In Fig. 1a the xenon has just begun to enter the lungs. In Fig. 1b the lungs are so inflated that they press against the interior of the 10-mm diameter glass tube in which they are contained. Finally, in Fig. 1c the lungs have started to deflate and the two lobes are clearly visible. The bright spot toward the centre is a cross-section of the trachea through which the xenon was introduced. All three ^{129}Xe images are dark in the region between the lung lobes where the heart is located. Comparison with a standard $^1\text{H}_2\text{O}$ image (Fig. 1d), in which the heart is prominent and the lungs invisible, illustrates one aspect of the complementary nature of proton- and ^{129}Xe -MRI. All ^{129}Xe images in Fig. 1 were obtained in 600 ms at an average lung Xe concentration of ~ 20 mM (1.2×10^{19} atoms cm^{-3}), which is tiny compared to the 80–100 M proton concentrations typical of $^1\text{H}_2\text{O}$ imaging; yet the signal intensities, spatial resolution (0.14–0.28 mm^3), and data acquisition rates all exceed those obtained in standard clinical $^1\text{H}_2\text{O}$ -MRI. Moreover, the magnetization densities are so large that several images can be generated in rapid succession, allowing for real-time tracking of physiological processes.

The large and long-lived ^{129}Xe magnetization densities achieved in the lung provide a basis for imaging beyond the lung itself—MRI of ^{129}Xe transported from the lung to various tissues is feasible. To assess the MRI parameters for ^{129}Xe in lipid environments we imaged a tube of octanol, a standard cell-membrane model, containing dissolved (205 mM) thermally-polarized xenon. The images were of lower resolution (20 mm^3) and required 7 min to obtain but clearly demonstrate the feasibility of using ^{129}Xe to image tissue. With hyperpolarized ^{129}Xe , high-resolution images comparable to those of Fig. 1 could be obtained in a single rapid imaging scan, even for the lower concentrations expected in tissue. In the brain, for example, a xenon concentration of ~ 10 mM is expected for a subject breathing 50% xenon¹⁵.

Imaging with a hyperpolarized species depends on the transport of sufficient surviving magnetization to tissues of interest. The polarization decays with the longitudinal relaxation time T_1 , which must be long in the lungs and blood. We have measured $T_1 \sim 30$ s for gaseous ^{129}Xe in the excised mouse lungs. Adjusting for the O_2 -relaxivity¹⁹ in a normally breathing subject we project values of 12–15 s in the lung. We also obtained a T_1 value of ~ 40 s for ^{129}Xe dissolved in partly deoxygenated rat blood. We estimate T_1 for ^{129}Xe in fully-oxygenated cell membranes to be >15 s (refs 15, 20, 21). This estimate must be compared to the time it takes for xenon to reach various tissues. Inhaled xenon reaches equilibrium with the entire blood volume in one blood circuit, (~ 1 s in a mouse, and ~ 15 s in a human^{9,22,23}). As transport and relaxation times are of the same order of magnitude, one can expect significant accumulation of highly polarized ^{129}Xe in all tissues.

The importance of this technique hinges on the radically

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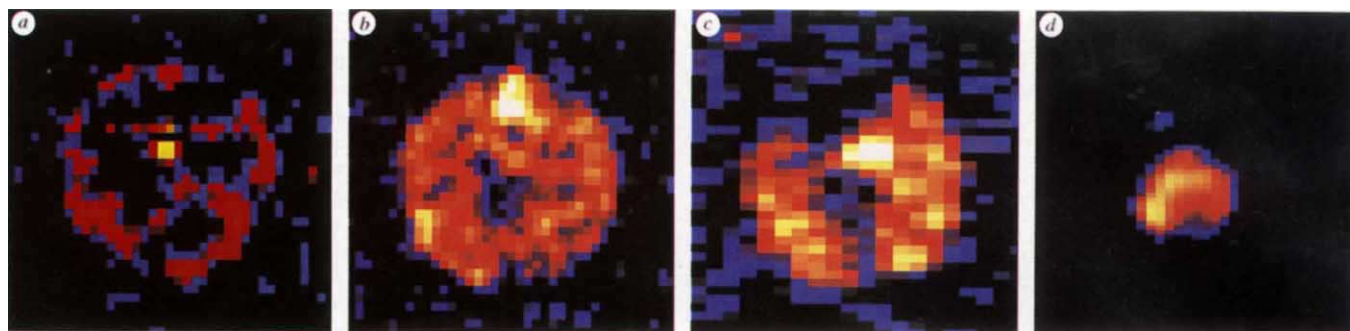


FIG. 1 Magnetic resonance images of the excised lungs and heart of a mouse as laser-polarized ^{129}Xe enters the lungs through the trachea: ^{129}Xe images, (a–c); $^1\text{H}_2\text{O}$ image, (d). All images are nominally of the same 1-mm-thick axial slice (perpendicular to the axis of the vertebral column), adjusted to the same scale: the lung outline in (a–c) is 10 mm in diameter. a, About 1 s after seal-breakage, *in situ* N_2 is still being displaced, and ^{129}Xe delivery is in progress. Probably only the larger air vessels are seen. Pixel resolution is $0.37\text{ mm} \times 0.37\text{ mm}$, image recorded in 600 ms. b, A second heart–lung preparation $\sim 2\text{ s}$ after seal breakage. Gas mixing in the fully-inflated lungs is complete. c, The same preparation 7 s later, imaged with the remaining ^{129}Xe hypermagnetization. The lungs are relaxed. Pixel size here is $0.37\text{ mm} \times 0.74\text{ mm}$, imaging time 300 ms. d, ^1H image of the same preparation. The bright heart fills the dark region of c. Note the absence of signal from lung-tissue $^1\text{H}_2\text{O}$, typical behaviour of regions with ultra-short T_2 when subjected to standard MRI. (Non-standard MRI can extract low-contrast signal from lung water, but images take 20–40 min (refs 1, 7) to acquire.) e, Diagram of the axial section of the preparation, corresponding to c and d, sketching the positions of the trachea, lung lobes, and heart.

METHODS. 3 atm of xenon gas, enriched to 70% ^{129}Xe , was laser-polarized as reported⁵, in glass cells containing a few mg Rb metal, and Rb vapour at $\sim 2 \times 10^{12}\text{ atoms cm}^{-3}$. After 20 min of irradiation the cell is seated in the delivery system. The polarized ^{129}Xe is transferred when a break-seal on the cell is ruptured with a piston. The Rb vapour cools,

different magnetic and physiological properties of ^{129}Xe and $^1\text{H}_2\text{O}$. In $^1\text{H}_2\text{O}$ -MRI, the small range of contrast arising from variations in local $^1\text{H}_2\text{O}$ concentration (proton-density contrast) is enhanced by exploiting the ~ 3 -fold variation of the longitudinal (T_1) and transverse (T_2) nuclear spin-relaxation times within normal and pathological tissues^{24,25}. The T_1 and T_2 variations for ^{129}Xe in lipid membranes, water, and proteins are much larger and more sensitive to chemical environment^{15,20,21}, particularly to O_2 concentration²¹. This broad range of ^{129}Xe T_1 and particularly of T_2 values should make it possible to distinguish between different tissue types. Further, the 20 p.p.m. range in resonance frequency of ^{129}Xe among the various tissue components^{14,15,18}, $\sim 200\text{ p.p.m.}$ away from the gas-phase resonance, may permit differential imaging of these components. In contrast, the resonance frequency of $^1\text{H}_2\text{O}$ in tissue is essentially invariant. Lastly, the hyperpolarized ^{129}Xe density in a tissue depends markedly on xenon transport. Changes in blood flow induced by physiological activity should produce straightforward changes in local hyperpolarized ^{129}Xe density contrast.

MRI with hyperpolarized ^{129}Xe already appears quite promising in comparison with other advanced imaging techniques. In lung imaging, for example, the only serious alternative to ^{129}Xe MRI is high-resolution X-ray computed tomography (HRCT). Typical resolutions of 4 mm^3 are obtained in 2 s but with a heavy radiation dose (1–5 rem) to the subject²⁶. With ^{129}Xe MRI one could obtain HRCT-like images of lung tissue density variation, using the gas-phase ^{129}Xe resonance, but one could also obtain images of the time-dependent dissolving ^{129}Xe distribution that reflects alveolar function. ^{129}Xe signal strength sufficient to image 4-mm^3 volume elements in a 0.6 s MRI FLASH scan would be obtained if 40 ml of pure 25% polarized ^{129}Xe (150 ml of natural Xe) were inhaled into the lung. Routine

condenses and is adsorbed onto the surfaces of the delivery system during transfer. Images were obtained using a Bruker MSL 400 instrument operating at 110.7 MHz for ^{129}Xe , with an 89 mm bore, 9.4 T vertical magnet and high-gradient Bruker microimaging probe. All images were obtained with a single Fast-Low-Angle-SHOT (FLASH^{30,31}) sequence and the same maximum encoding field gradients (resulting in a field of view of $47\text{ mm} \times 47\text{ mm}$ for the ^{129}Xe images, and $13\text{ mm} \times 13\text{ mm}$ for ^1H), using either 128×128 (a, b) or 64×128 matrices (c, d).

production of such quantities is currently practical. The low cost of Xe gas ($\sim \$15$ – 20 per litre in bulk for natural-abundance Xe) will permit imaging at higher resolution of lung and of other tissues, which requires inhalation of larger volumes of hyperpolarized ^{129}Xe . If desired, Xe, which is not metabolized, can readily be recovered from the exhaled gas. Like standard $^1\text{H}_2\text{O}$ imaging, ^{129}Xe MRI should be a safe procedure.

Thus laser-polarized ^{129}Xe has the potential to add significantly to the scope of MRI. We note that ^3He is also an attractive candidate for imaging; it can be produced with comparable hyperpolarization, and has a larger magnetic moment and longer relaxation times²⁷. The prospect of scaling-up hyperpolarized noble-gas technology to human subjects, and all tissues, is promising. Although this extension will require larger quantities of hyperpolarized ^{129}Xe than those used in a mouse, the much larger volume elements ordinarily imaged will tolerate lower concentrations. A single commercially available Ti:sapphire laser system can in principle produce about 1 g h^{-1} of highly polarized ^{129}Xe (ref. 28), which can be stored frozen for hundreds of hours without substantial loss of polarization²⁹. We believe the way is open to ^{129}Xe imaging of any desired portion of a living mammal. □

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Radiocarbon evidence for a smaller oceanic carbon dioxide sink than previously believed

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RADIOCARBON produced naturally in the upper atmosphere or artificially during nuclear weapons testing is the main tracer used to validate models of oceanic carbon cycling, in particular the exchange of carbon dioxide with the atmosphere^{1–3} and the mixing parameters within the ocean itself^{4–7}. Here we test the overall consistency of exchange fluxes between all relevant compartments in a simple model of the global carbon cycle, using measurements of the long-term tropospheric CO_2 concentration⁸ and radiocarbon composition^{9–12}, the bomb ^{14}C inventory in the stratosphere^{13,14} and a compilation of bomb detonation dates and strengths¹⁵. We find that to balance the budget, we must invoke an extra source to account for 25% of the generally accepted uptake of bomb ^{14}C by the oceans³. The strength of this source decreases from 1970 onwards, with a characteristic timescale similar to that of the ocean uptake. Significant radiocarbon transport from the remote high stratosphere and significantly reduced uptake of bomb ^{14}C by the biosphere can both be ruled out by observational constraints. We therefore conclude that the global oceanic bomb ^{14}C inventory should be revised downwards. A smaller oceanic bomb ^{14}C inventory also implies a smaller oceanic radiocarbon penetration depth¹⁶, which in turn implies that the oceans take up 25% less anthropogenic CO_2 than had previously been believed.

The atmospheric $^{14}\text{CO}_2$ activity has undergone large excursions since the beginning of nuclear bomb tests (Fig. 1a and b, solid lines). After the Test Ban treaty in 1962 the bomb ^{14}C signal in the atmosphere is declining because of $^{14}\text{CO}_2$ exchange with the ocean and the other carbon reservoirs. The behaviour of these ^{14}C exchange fluxes over time depends mainly on the total carbon fluxes between the reservoirs, and on the internal circulation dynamics within these reservoirs.

The temporal variation of the tropospheric radiocarbon inventory N_{trop} is determined by the net exchange fluxes with the ocean F_{O} , the terrestrial biosphere F_{B} , and the stratosphere F_{S} , by input from anthropogenic sources Q_{Trop} , and the radioactive decay (^{14}C has a mean lifetime $\lambda^{-1} = 8,275$ yr) as follows

$$d(N_{\text{trop}})/dt = F_{\text{O}} + F_{\text{B}} + F_{\text{S}} + Q_{\text{Trop}} - \lambda N_{\text{trop}}$$

Only the global response on the interannual timescale to a major atmospheric perturbation is examined in this study. We can therefore use relatively simple models to determine the respective radiocarbon fluxes. For the ocean, a type of robust Oeschger and Siegenthaler box diffusion model^{4,5} was adopted using a vertical eddy diffusion coefficient $K = 7,685 \text{ m}^2 \text{ yr}^{-1}$ coupled to 7.8 yr residence time of atmospheric CO_2 with respect to air/sea gas exchange. The flux F_{O} , calculated according to our tropospheric boundary conditions, matches the integrated oceanic bomb ^{14}C uptake (until 1 January 1974) of 300×10^{26} atoms (Fig. 2a) derived from oceanic measurements during GEOSECS³, and compares well with results of the most recent version of the HILDA¹⁷ ocean model.

The model biosphere is divided into three boxes where the input carbon is decomposed exponentially with an e-folding constant given by the turnover time τ . Box 1 has a mass of 105 Gt-C (gigatonnes carbon; $1 \text{ Gt} = 10^{15} \text{ g}$), $\tau = 3$ yr, and accounts for fine roots, twigs and leaves. Box 2 has a mass of 675 Gt-C, $\tau = 27$ yr, and represents big roots, stems and branches. Boxes 1 and 2 couple directly to the troposphere, and the sum of their input fluxes, determining the net primary productivity, is set to 60 Gt-C yr^{-1} . Box 3, the 'old carbon reservoir' has a mass of 1,420 Gt-C and $\tau = 375$ yr. Box 3 contains the slowly decomposing material of boxes 1 and 2, gets its carbon input equally distributed from these boxes, and is needed to account for the low $\Delta^{14}\text{C}$ values measured in soil organic carbon¹⁸. These settings correspond to previously published estimates for the terrestrial biosphere¹⁹. We did not account for fertilisation and destruction fluxes when calculating F_{B} from the tropospheric boundary conditions.

Our model stratosphere consists of one box with the same CO_2 concentration as the model troposphere, and a turnover time of 2.5 yr with respect to the troposphere. The total mass of the stratosphere box corresponds to 15% of the total atmosphere air mass. F_{S} was calculated from the bomb input scenario and the measured tropospheric boundary conditions.

The initial conditions in 1945 for all reservoirs were computed starting at preindustrial equilibrium in AD 1750 (atmospheric concentrations: 280 p.p.m.v. CO_2 , $\Delta^{14}\text{C} = -4.5\text{‰}$). Using observed atmospheric CO_2 concentrations⁸ and $^{14}\text{CO}_2$ data^{9,12} (Fig. 3b) as prescribed input data in all scenarios, we automatically account for the dilution of $^{14}\text{CO}_2$ by input of ^{14}C free carbon from fossil fuel consumption (Suess effect²⁰). All natural ^{14}C production ($P_{\text{nat}} = 2.3 \times 10^{26} \text{ atoms yr}^{-1}$, assumed to be constant) occurred in the stratosphere.

The anthropogenic input of $^{14}\text{CO}_2$ by the nuclear industry, contributing significantly to the tropospheric inventory only from about 1970 onwards, was calculated for different reactor types using the normalized $^{14}\text{CO}_2$ emission data per generated electrical energy reported by Bonka²¹ and in UNSCEAR²². The latter was estimated for the period of 1970 to 1990 from the installed plants worldwide, assuming a capacity utilization of 60% for all reactor types. $^{14}\text{CO}_2$ emissions from reprocessing plants were also taken from UNSCEAR²². The $^{14}\text{CO}_2$ release from the nuclear industry in 1990 was estimated to be less than $0.5 \times 10^{26} \text{ }^{14}\text{C-atoms yr}^{-1}$, increasing almost linearly from 1970 onwards (compare Fig. 2b).

^{14}C input from the atmospheric bomb tests was estimated based on the compilation of bomb strength data¹⁵ (Fig. 1b), and, depending on the respective scenario, adjusting the specific ^{14}C production per megatonne (Mt) TNT to the tropospheric and stratospheric observations during the time period of the major ^{14}C rises. The uncertainty of this adjustment is small as

FIG. 1 *a–d*, Comparison between results from two ^{14}C model scenarios (dotted lines) and observations (solid lines) in the stratosphere (*a* and *c*), and in the troposphere (*b* and *d*). The observed stratospheric inventories were taken from Tans¹³ and Telegadas¹⁴ (according to Tans¹³, the original observations are corrected by -20% ; a further adjustment of $+3.5\%$ was made to correct for the NBS oxalic acid standard activity value used by Telegadas¹⁴). Mean tropospheric ^{14}C inventories are calculated from long-term tropospheric observations in both hemispheres^{9–12}. For the early period of 1950–59, we use tree ring ^{14}C data³³. In both scenarios, the stratosphere consists of only one box with an air mass of 15% of the atmosphere, corresponding to a tropopause level at 13.5 km. In scenario I (*a* and *b*), the bomb ^{14}C input is estimated using the bomb strength data (*b*), and the standard ^{14}C yield P_{stand} of 1.75×10^{26} atoms per Mt-TNT (ref. 23). With P_{stand} the tropospheric and the stratospheric $^{14}\text{CO}_2$ levels are overestimated. In scenario II (*c* and *d*) the bomb ^{14}C input is adjusted to 60% of P_{stand} . The model matches the observations in the troposphere until about 1963. After that date the decrease in the troposphere is much faster than actually observed. The missing tropospheric $^{14}\text{CO}_2$ source needed to adjust model and data results is similar in time-dependence and strength to 25% of the oceanic or, equivalently, to 80% of the biospheric net bomb ^{14}C uptake flux (see Fig. 3e and *d*).

the total bomb ^{14}C uptake by the ocean and the biosphere, compared to the bomb input, is small until 1963. As the observational data in the troposphere show a systematic delay between the date when the stronger bombs were fused, and the date when the respective signal showed up in the troposphere, we introduced all bomb ^{14}C production directly into the stratosphere. The results reported in Fig. 1*a* and *b* show clearly that the ^{14}C production calculated with the standard ^{14}C yield $P_{\text{stand}} = 1.75 \times 10^{26}$ atoms per Mt-TNT (ref. 23) was overestimated. In fact, estimates of P_{stand} are in the range $(1–2) \times 10^{26}$ atoms per Mt-TNT (refs 21, 24).

Figure 1*c* and *d* (scenario II) shows the results obtained when reducing the value of P_{stand} by 40%. The model inventory fits well with the data in the stratosphere and in the troposphere until 1963, as long as the bomb production is the dominant flux term. In the post-bomb period, the model troposphere is influenced by a much too strong sink term. The fictive tropospheric source needed to adjust the model and data turns out to be similar in time-dependence and strength to 25% of the oceanic or, equivalently, to 80% of the biospheric net bomb ^{14}C uptake flux (Fig. 2*b*). The magnitude of the missing source can be in error by at most one-third. This is mainly due to the strong constraint on the coupling constants between the reservoirs given by the >40 -yr record of tropospheric $^{14}\text{CO}_2$ observations. The strength of the missing source should decrease with an e-folding time similar to the ocean uptake, that is, ~ 8 yr. Neither the nuclear industry nor the natural cosmic ray production can account for this source. The variation of cosmic ray production is $\sim 40\%$ between solar minimum and solar maximum²⁵, and thus at least one order of magnitude too small. The ^{14}C production by nuclear industry is only a few per mil of the needed ^{14}C source in the 1970s, while increasing instead of declining (Fig. 2*b*).

Looking for a candidate in the atmosphere itself, we divided the stratosphere into two boxes. The high box contains $<1.5\%$ of the atmospheric mass (lower boundary corresponding to 25 km a.s.l.) and has a turnover time τ of 5 yr exchanging with the low box ($\tau = 2.5$ yr with respect to the troposphere). In Fig. 3*a* and *b* the results obtained with such a two-box stratosphere (scenario III) are shown. In this scenario, 15% of the adjusted bomb production (in this case 70% of P_{stand}) is injected into the high stratosphere, the rest into the low stratosphere. Scenario III now leads to a nearly perfect agreement between the model and the observations in the lower stratosphere and in the tropo-

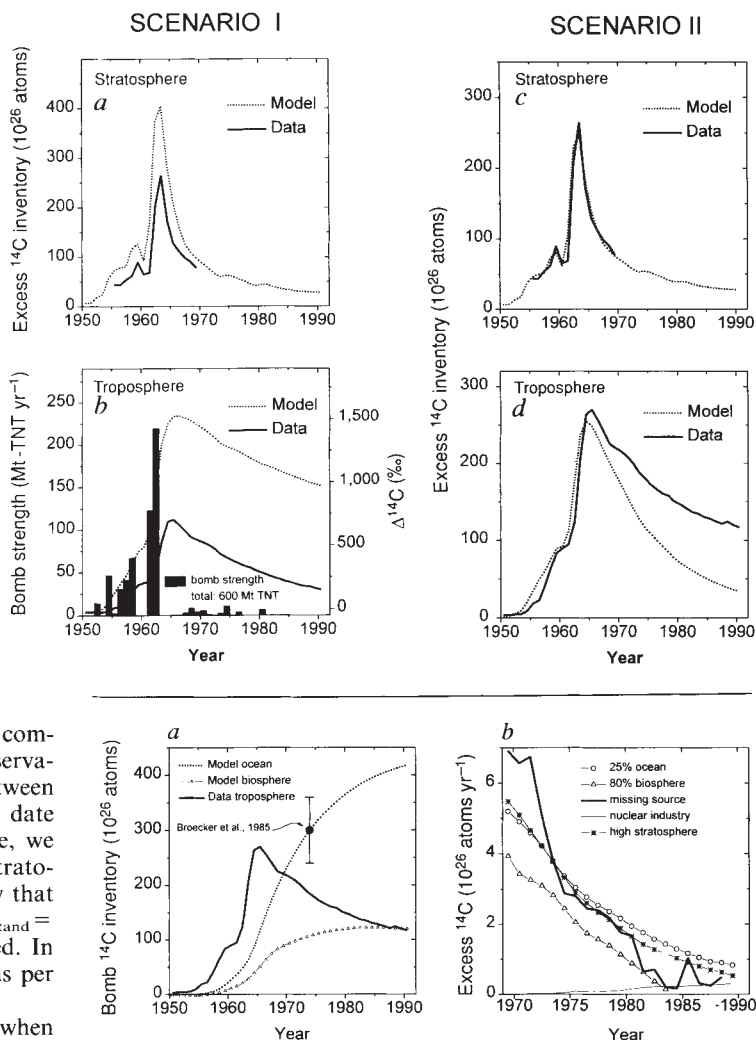
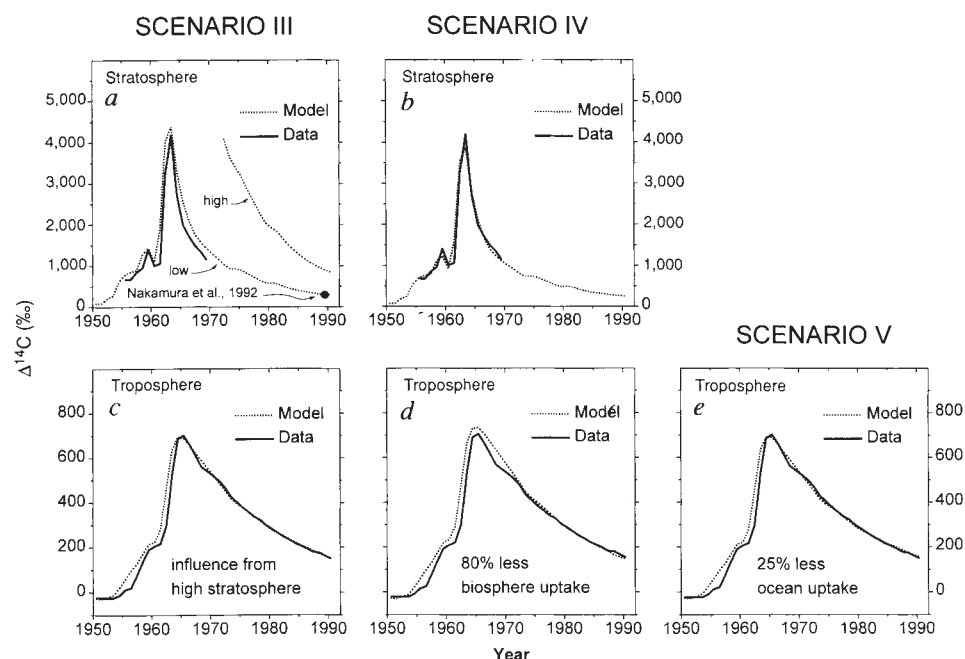


FIG. 2 Bomb ^{14}C inventories (*a*) and missing source flux (*b*). *a*, The standard bomb ^{14}C inventories are calculated as the difference to the respective ^{14}C inventory in 1940. The standard inventories for the ocean (dotted line) and the biosphere (Δ) are as calculated for scenarios I, II and III with prescribed tropospheric values. The standard ocean bomb ^{14}C inventory on January 1974 matches the value of 300×10^{26} atoms ($\bullet$) given by Broecker et al.³. *b*, The missing ^{14}C flux to the troposphere (6 year running means, thick solid line) is as calculated in scenario II (Fig. 1*d*). This missing flux is compared to the net supplementary contributions from (1) a high stratosphere ($\star$, scenario III), (2) a 80% reduction of the standard biospheric uptake (Δ , scenario IV), and (3) a 25% reduction of the standard oceanic uptake ($\circ$, scenario V). In all three cases, the supplementary flux has the right time-dependence and amplitude to account for the missing source (Fig. 3*b*, *d* and *e*). The emission from nuclear installations (thin solid line) is also given for comparison.

sphere. However, scenario III demands very high $\Delta^{14}\text{C}$ values in the remote high stratosphere (Fig. 3*a*) which is inconsistent with recent $^{14}\text{CO}_2$ observations²⁶ up to 30 km height. Also the old data from Telegadas¹⁴ obtained in the early 1960s suggest a $\Delta^{14}\text{C}$ decrease rather than an increase to higher stratospheric levels. Moreover, the observed decrease from intermediate to high stratospheric levels has also been obtained by recent high-resolution stratospheric model calculations²⁷. Therefore even a supposed remote stratosphere with still very high $^{14}\text{CO}_2$ can most probably not close the bomb ^{14}C budget.

On the other hand, assuming almost no bomb ^{14}C uptake by the terrestrial biosphere (scenario IV, Fig. 3*c* and *d*) would also match the bomb radiocarbon constraints. In our scenario this reduced uptake is simply obtained by multiplying the standard

FIG. 3 Comparison between observed and calculated $\Delta^{14}\text{C}$ values in the troposphere (b, d, e) and in the two- and one-box stratosphere (a and c respectively). ^{14}C results of stratospheric sampling in 1989 (ref. 26) are included in a. ($\Delta^{14}\text{C}$ concentrations are per mil deviations from NBS oxalic acid activity corrected for decay³⁴). In scenario III (a and b) bomb ^{14}C input is adjusted to 70% of P_{stand} . The stratosphere is subdivided into two boxes, 85% of the bomb ^{14}C input is introduced into the lower stratosphere, and 15% into the remote high stratosphere. The model results agree well with the atmospheric observations in the troposphere and in the lower stratosphere (90% of stratospheric air mass, 13.5–25 km height). The stratospheric observations, however, indicate that, even in the early 1990s, the modelled upper stratospheric $\Delta^{14}\text{C}$ is still ~ 2 times too high. In scenario IV (c and d) and V (e), as in scenario II (Fig. 1c and d), bomb ^{14}C input is adjusted to 60% of P_{stand} and introduced into the one-box stratosphere. In scenario IV, the bomb ^{14}C uptake by the biosphere has been reduced by 80%, and in scenario V, bomb ^{14}C uptake by the oceans has been reduced by 25% with respect to the standard case given in scenario II. Scenarios IV and V satisfactorily match the $^{14}\text{CO}_2$ observations, both in



uptake of the biosphere by 0.2. In reality, such a strong reduction is only achieved if, for example, the net primary productivity is reduced by a factor of 5 and the reservoir sizes are modified accordingly. This, however, would so seriously contradict our understanding of mass, cycling and turnover times in the biosphere²⁸ that scenario IV appears highly improbable.

The most tempting solution to the problem would be an $\sim 25\%$ reduction of the ^{14}C uptake by the oceans (scenario V, Fig. 3c and e) leading to an oceanic bomb ^{14}C inventory reduced by the same amount. This means only a correction to known processes (for example, gas exchange rate and vertical mixing) rather than introducing, for example, still unconsidered sub-reservoirs as in the biospheric or stratospheric scenarios. However, a 25% reduction of the bomb ^{14}C inventory of the oceans lies outside the error bars generally accepted for this quantity (20%, ref. 3). The contradiction gets even larger when taking into account the very recent upward revision of the oceanic bomb ^{14}C inventory, evaluated on the basis of more observations and an improved estimation of the pre-bomb natural oceanic radiocarbon distribution²⁹. This problem needs to be resolved.

A 25% reduction of bomb ^{14}C uptake by the ocean models would have significant implications for our understanding of the global carbon cycle. First, the radiocarbon-derived CO_2 gas exchange coefficient has to be reduced by the same amount, then being almost in agreement with the estimates of Liss and Merlivat³⁰, which were based on direct measurements in wind tunnels and over lakes and open ocean³¹. Second, if we believe the ^{14}C observations in surface water performed during the past 30 years, the estimated bomb ^{14}C penetration depth³² has to be reduced. Furthermore, if it is assumed that the CO_2 uptake by the oceans scales directly with the bomb ^{14}C penetration depth¹⁶, a downward revision of the latter would imply a corresponding reduction by $\sim 25\%$ of the inferred oceanic sink for anthropogenic CO_2 . □

the troposphere as well as in the one-box stratosphere (the stratospheric model results of scenario V are indistinguishable from those of scenario IV). However, neither of these scenarios corresponds to our present understanding of the carbon cycle.

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Forecasting Zimbabwean maize yield using eastern equatorial Pacific sea surface temperature

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SOUTHERN Africa is subject to recurrent droughts which cause severe food shortages. There is considerable evidence¹ that El Niño² warm events in the Pacific Ocean are linked to below-average rainfall in southern Africa, and the 1991–92 El Niño event was accompanied by the worst drought in southern Africa this century, affecting nearly 100 million people. But although models can predict El Niño events a year in advance^{3–6}, the drought was not anticipated, increasing relief costs. Here we present data showing a strong correlation between an El Niño index and both rainfall and maize yield in Zimbabwe. Surprisingly, the correlation with maize yield is stronger than that with rainfall, with more than 60% of the variance in yield accounted for by sea surface temperatures in the eastern equatorial Pacific Ocean—half-way around the world. We also show that model predictions of the El Niño index provide accurate forecasts of maize yield in Zimbabwe, with lead times of up to a year. As maize is the most important food crop for the ten-nation Southern African Development Community region⁷, we suggest that this approach could provide an effective early-warning system for southern African drought-induced famines.

Maize is by far Zimbabwe's most significant food source⁷. Figure 1 (solid lines) shows annual variations in Zimbabwe rainfall and national average maize yield for the period 1970–93. Earlier data on the maize crop are unreliable. The annual average rainfall is derived by summing monthly weighted averages of rain gauge reports. Throughout the period under review there were ~1,150 stations spread fairly evenly throughout the country.

Before independence in 1980, the bulk of the marketed maize came from large-scale commercial farms. Since 1980, however, the Grain Marketing Board has stimulated a marked increase in the volume grown and marketed by small-scale, communal farmers and in the overall area sown to maize. The overwhelming bulk of maize produced is now rain-fed. Adoption of hybrid varieties has also been widespread. This has resulted in a slight trend towards an increase in overall yields, but has also contributed to wider differences in yields between good and poor years. Temperature and the distribution of rainfall during the growing season influence plant growth. Maize needs moisture during inflorescence for the pollen to stick to the tassels and, later, to fill the grain. January to March is the critical period. Despite additional climate and economic influences on the maize crop, it is evident in Fig. 1 that fluctuations in average yields clearly follow those in annual rainfall. The strength of this connection is clearly demonstrated by the variations in recent years. Average yields reached a high of 2.4 tonnes per hectare ($t\ ha^{-1}$) in 1986, and a low of $0.4\ t\ ha^{-1}$ in 1992. What is of more interest, however, is that yields also track changes in an index of El Niño Southern Oscillation (ENSO).

Figure 1 (dashed lines) shows an index of ENSO, scaled to be in the same units as the corresponding Zimbabwe variables. Specifically, the index is the sea surface temperature (SST) anomaly in the NINO3 region (90° – 150° W, 5° S– 5° N) of the eastern equatorial Pacific. This primary manifestation of ENSO is widely monitored, and is reported monthly in the *Climate Diag-*

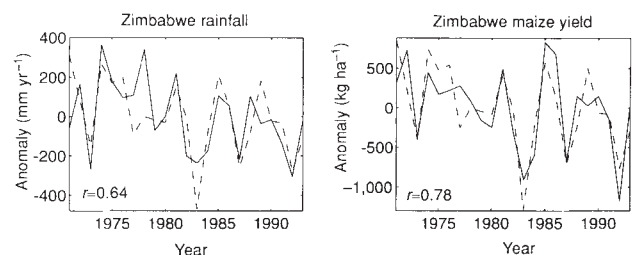


FIG. 1 Solid lines, annual variations in Zimbabwe rainfall (left) and maize yield (right) for the period 1970–93 ('1970' denotes the 1969–70 growing season, November 1969 to May 1970). Yield data were collected by the extension service of the Zimbabwe Ministry of Lands, Agriculture and Water Development. Dashed lines, SST anomalies in the NINO3 region of the eastern equatorial Pacific (see text). r is the correlation coefficient of the two curves.

nostics Bulletin produced by the Climate Analysis Center of the US National Weather Service. We prefer it as an ENSO index to the still more widely used Southern Oscillation Index (SOI) because it is far less noisy; that is, it has less month-to-month variability due to atmospheric fluctuations distinct from ENSO.

The dashed lines of Fig. 1 are for the NINO3 index averaged for February and March, the heart of the growing season. The strength of the correspondence between rainfall in Africa and the surface temperature of the ocean almost half-way around the world is astonishing: the correspondence between NINO3 and the maize yield is even better. (There is only one chance in five that the difference between yield and rainfall is accidental: the correlation coefficient r of NINO3 is 0.64 with rainfall and 0.78 with maize yield; according to the standard F -test for correlation coefficients, the difference is significant at the 82% level for a sample of size 23.) It may be that fluctuations in maize yield are amplified by rainfall variations in the drier parts of the country. The spread of hybrids is an additional, nation-wide amplifier. On the other hand, the stronger connection of NINO3 SST to maize yield may be due to the other climate factors influencing plant growth.

Because the NINO3 SST values of Fig. 1 are simultaneous with the growing season, their predictive value is limited. The solid curves in Fig. 2 show the correlations of rainfall and maize yield with observed NINO3 SST at all lead times beginning with the January of the previous growing season (that is, more than 1 year in advance). Correlations are still fairly high in the autumn preceding the growing season, but decrease rapidly as the lead time lengthens.

To extend the lead time for prediction, we use a simulation model able to predict variations in the tropical Pacific associated with ENSO. The model we use, that of Zebiak and Cane^{8,9}, has been used to predict ENSO since 1985, and its performance is

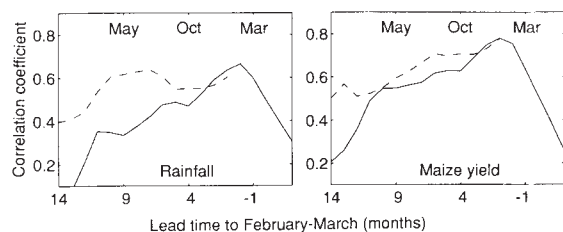


FIG. 2 Solid lines, correlation coefficients of rainfall (left) and maize yield (right) with observed NINO3 SST at all lead times beginning with the January of the previous growing season (that is, more than 1 year in advance). Dashed lines, correlation coefficients with the observed rainfall (left) and maize yield (right) obtained for forecasts from each calendar month using the ENSO prediction model of Zebiak and Cane^{8,9}.

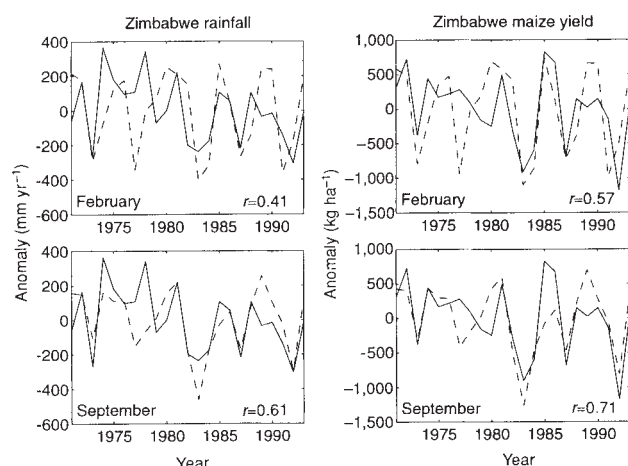


FIG. 3 As Fig. 1, but here it is the model-derived forecasts from the preceding February and September that are compared with the observed values.

well documented³⁻⁶. The forecast scheme we adopt is the following. Given the data needed to initialize the model for a particular month, we use it to forecast the NINO3 SST anomalies for the following February–March. The forecast NINO3 value is then used as a predictor for the rainfall or maize yield.

The dashed lines in Fig. 2 show the correlation with the growing season rainfall and maize yield obtained for forecasts from each calendar month. In contrast to the results for the observed NINO3, there is little fall-off in correlation as the lead time increases. Longer lead forecasts are now possible. Figure 3 is similar to Fig. 1, but now the forecasts from the preceding February and September are compared with the observed values. Of course, the correlations are not as high as for the values of NINO3 actually observed during the growing season, but they are high enough to give significant skill. (For the less skilful February forecast the correlation with rainfall is 0.41, significant at the 99.5% level, whereas that with maize yield is 0.57, significant at the 99.9% level. For the September forecasts the corresponding correlations are 0.61 and 0.71.) The February forecast anticipates maize yield in Zimbabwe a full year ahead—before the previous harvest is in. The September forecast could be available in October, before the time for planting.

Obviously, the possible prediction schemes are far from exhausted. For example, the year-to-year change of the SOI has been used as a predictor of the Australian wheat crop¹⁰. However, in view of the shortness of the maize time series, we prefer to stop at a point where our prediction procedures are so straightforward that there can be little question of artificial skill (that is, the danger that in a short record a random coincidence of variations will be mistaken for a true relation between the variables). The high correlations already obtained leave little room for improvement by technical elaboration. Figure 1 suggests that better ENSO predictions are a primary requirement for substantially improved maize forecasts. Additional improvement would require the use of more localized factors as predictors. It would be especially desirable to go beyond national crop yields to regional forecasts.

As noted above, the 1991–92 drought in southern Africa was the worst this century. The Southern African Development Community (SADC) region's cereal harvest was halved and nearly 100 million people living in the 10 SADC states and South Africa were affected. Fortunately, widespread famine was averted by responsive actions by local governments and aid from the international community.

This successful response is all the more notable because the drought was not anticipated (though the ENSO warm event

was¹¹). Regional grain stocks were known to be low before the growing season, but good rains in October and November 1991 prompted optimism instead of corrective action. Consequently, the subsequent relief effort was more costly and more precarious. Ultimately, some 11.6 million tons of drought-related commodities had to be imported within a 13-month period¹², a considerable logistic achievement.

We believe that the results reported here indicate that long-term forecasts, although hardly infallible, are sufficiently reliable to be useful inputs to the SADC Regional Early Warning System. ENSO forecasting models can provide indications of probable average crop yields even before the previous harvest is in. As the growing season approaches the forecasts become more skilful, and as it begins, estimates can be further sharpened by folding in information on the current ENSO state. It is both scientifically noteworthy and fortunate that the stronger connection is not to rainfall but to maize yield, one link closer to the food supply.

The ENSO cycle will surely continue, and there will surely be another drought threatening the welfare of the people of southern Africa. An effective early warning system is in place, and relevant climate knowledge continues to grow. Next time we will be better able to anticipate and react early to minimize the cost and the damage to the community. □

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Origin of the slab component in arc lavas from across-arc variation of B and Pb isotopes

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AT convergent margins, the subducting oceanic slab is thought to dehydrate, producing fluids which metasomatize the overlying mantle wedge where island-arc magma forms. However, the nature and origin of the metasomatizing fluid, its source composition and its relation to the genesis of the chemical characteristics of arc magmas are largely controversial. Across-arc variation in the chemistry of arc lavas provides a useful key to this problem, because it may reflect the changes in the physical conditions of the subducting slab that control mass transfer from slab to mantle wedge as a function of depth. Here we report clear across-arc variations in the concentrations and isotopic compositions of boron

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and lead observed in lavas from the Izu arc (Japan). Our data suggest that a homogeneous slab fluid contributes to all Izu volcanoes, but that the amount of this fluid decreases continuously with increasing depth of the subducting slab. Whereas the Izu slab fluid comes primarily from altered oceanic crust, our data for high-Mg andesites from the Setouchi volcanic belt (a nearby fore-arc) indicate a significant involvement of sediment in the fluid source.

Boron is an excellent tracer of crustal recycling at subduction zones^{1,2}, because it is highly concentrated in subducting sediments and altered oceanic crust (AOC) relative to the overlying mantle wedge, and is extremely mobile during both partial melting and dehydration. Although B isotopic compositions ($\delta^{11}\text{B}$) of terrestrial rocks have not been well established, a few pioneering studies suggested that B isotopes could reflect subduction components in arc lavas^{3,4}. Recently we found that the $\delta^{11}\text{B}$ values of subducting sediment and AOC are different⁵, an observation that enhances the potential of using B isotopes to understand the processes occurring at subduction zones.

In the Izu arc lavas (Fig. 1), clear across-arc variations are observed in B/Nb, Pb/Nb, $\delta^{11}\text{B}$ and Pb isotope ratios, which are all highest at the trench side and systematically decrease with increasing depth of the Wadati-Benioff zone (WBZ) (Table 1, Fig. 2). The B/Nb of 40, Pb/Nb of 6 and $\delta^{11}\text{B}$ of +7‰ found in the samples nearest the trench are significantly higher than those of mid-ocean-ridge basalt (MORB) (B/Nb < 1, Pb/Nb < 0.2 and $\delta^{11}\text{B}$ = -4 to 0‰)^{6,9} and ocean island basalt (OIB) (B/Nb < 0.1 and Pb/Nb < 0.2, $\delta^{11}\text{B}$ not given)^{9,10}. On the other hand, the values of these indicators in samples further from the trench approach those of MORB.

Because granites and sedimentary rocks generally have $\delta^{11}\text{B}$ < -2‰ (refs 5, 11), assimilation of these B-rich crustal rocks is not a process controlling the B/Nb and $\delta^{11}\text{B}$ of the Izu arc lavas. B and Nb have nearly identical solid/melt bulk distribution coefficients under upper-mantle and crustal conditions^{8,10}, so that the large variation in B/Nb cannot be produced by varying degrees of partial melting of the mantle and/or fractional crystallization of the magma. The depletion of high-field-strength elements (HFSE) such as Nb, Ta, Ti and Zr observed in arc magma had been hypothesized to result either from residual titanite phase in the mantle source¹² or from mantle-magma interaction¹³, which could cause the observed B/Nb variation. However, the linear relationship between Nb/B and $\delta^{11}\text{B}$ (Fig. 3a) is inconsistent with these ideas because the above HFSE depletion processes cannot coherently affect the B isotopic composition of arc magma. Alternatively, this relationship can be easily explained by simple mixing between two chemically homogeneous endmembers which have distinct B/Nb and $\delta^{11}\text{B}$ values. Although Nb is slightly more incompatible than Pb⁹, a tight linear correlation is also observed between Nb/Pb and $^{207}\text{Pb}/^{204}\text{Pb}$ (Fig. 3b). All the above observations strongly suggest that hydrous fluid from the subducting slab enriched in H₂O-soluble elements such as B and Pb extensively metasomatizes originally depleted MORB-like mantle wedge beneath the Izu arc, leaving behind H₂O-insoluble HFSE in the dehydrated slab. Assuming that Nb is completely immobile during dehydration of the slab, B and Pb isotopic compositions of endmember fluid can be estimated by extrapolating the linear trends in Fig. 3 to Nb/B = 0 and Nb/Pb = 0, which yields $\delta^{11}\text{B}$ = +7.4 ± 0.4‰ and $^{207}\text{Pb}/^{204}\text{Pb}$ = 15.561 ± 0.002.

Thus the across-arc variations observed in B/Nb and Pb/Nb (Fig. 2a) reflect the change in degree of metasomatism in the mantle wedge by slab-derived fluid. It further suggests that the amount of hydrous fluid is most abundant at the volcanic front and continuously decreases with increasing depth of the WBZ, possibly reflecting the progressive loss of volatiles in the subducting slab. The B-metasomatism beneath Kozushima (depth of WBZ, 210 km) is inferred to be only one-fifteenth of that beneath Ohshima (depth of WBZ, 150 km). The continuous supply of fluid observed here is obviously in conflict with the model of stepwise liberation of fluid resulting from the breakdown of

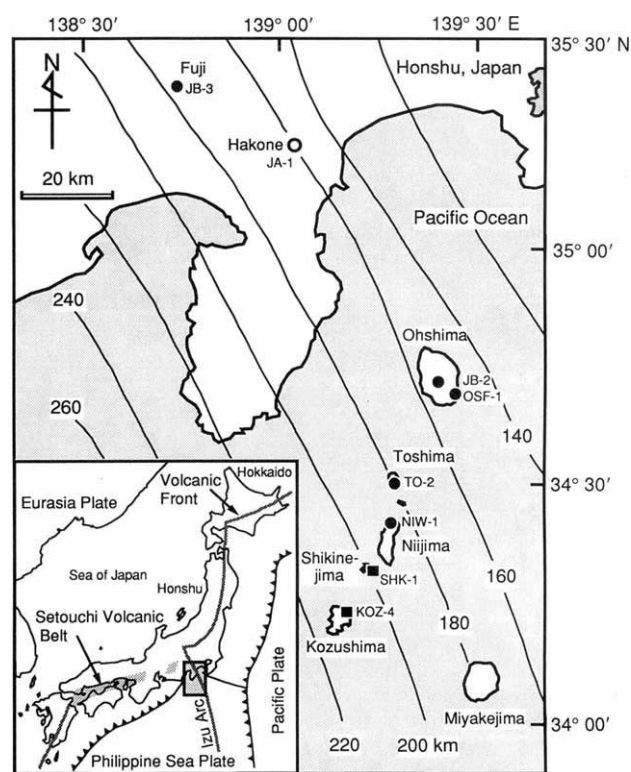


FIG. 1 Sample localities and the Wadati-Benioff zone around the Izu arc in Japan. This region is the northernmost part of the Izu-Mariana intra-oceanic island arc where the Pacific plate is subducting underneath the Philippine Sea plate. Contours represent isobaths for the top of the deep seismic zone (Wadati-Benioff zone) caused by subduction of the Pacific plate³¹. The analysed Quaternary volcanic rocks include basalt and basaltic andesite (filled circles), andesite (open circle) and rhyolite (filled squares). The volcanic centres of Hakone, Ohshima and Miyakejima represent the volcanic front in this region.

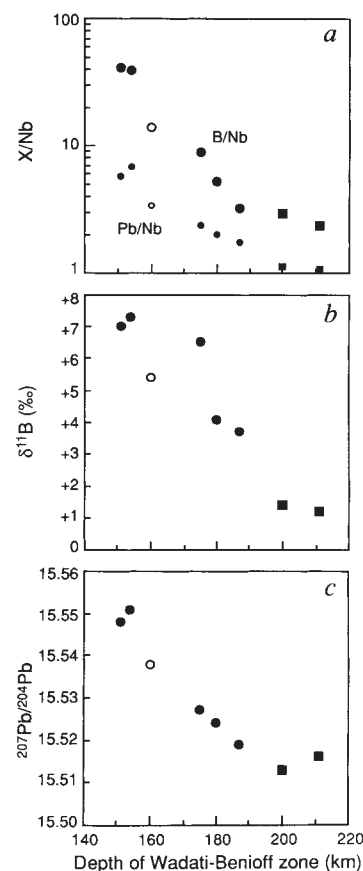


FIG. 2 The depth of the Wadati-Benioff zone plotted against B/Nb and Pb/Nb (a), $\delta^{11}\text{B}$ (b) and $^{207}\text{Pb}/^{204}\text{Pb}$ (c) of the Izu arc lavas. The symbols used for the lavas are the same as FIG. 1.

TABLE 1 Major- and trace-element contents and isotopic compositions of rocks

Sample	SiO ₂ (wt%)	MgO (wt%)	B (p.p.m.)	Pb (p.p.m.)	Nb (p.p.m.)	$\delta^{11}\text{B}$ (‰)	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$
Izu arc lavas									
OSF-1	49.30	4.64	8.25	1.16	0.2	+7.0	18.423	15.548	38.334
JB-2	53.20*	4.66*	31.2	5.4*	0.8*	+7.3	18.334	15.551	38.245
JA-1	64.06*	1.61*	23.8	5.8*	1.7*	+5.4	18.303*	15.538*	38.242*
JB-3	51.04*	5.20*	20.3	5.5*	2.3*	+6.5	18.287*	15.527*	38.208*
TO-2	51.13	7.24	6.77	2.58	1.3	+4.1	18.272	15.524	38.211
NIW-1	50.33	5.06	2.91	1.55	0.9	+3.7	18.316	15.519	38.188
SHK-1	77.74	0.01	17.2	6.56	5.8	+1.4	18.256	15.513	38.147
KOZ-4	76.23	0.25	12.9	5.79	5.4	+1.2	18.262	15.516	38.143
Setouchi high-Mg andesites									
TGI	58.50*	9.47*	30.7	11.4		-6.8	18.442	15.585	38.612
SD-264	58.42*	6.05*	64.5	16.3		-6.3	18.384	15.599	38.601
Shimanto shales									
S-605			123*	29.9		-5.6*	18.430	15.592	38.641
S-606			44.9*	21.4		-8.4*	18.394	15.584	38.677
S-607			131*	13.4		-7.9*	18.367	15.574	38.589

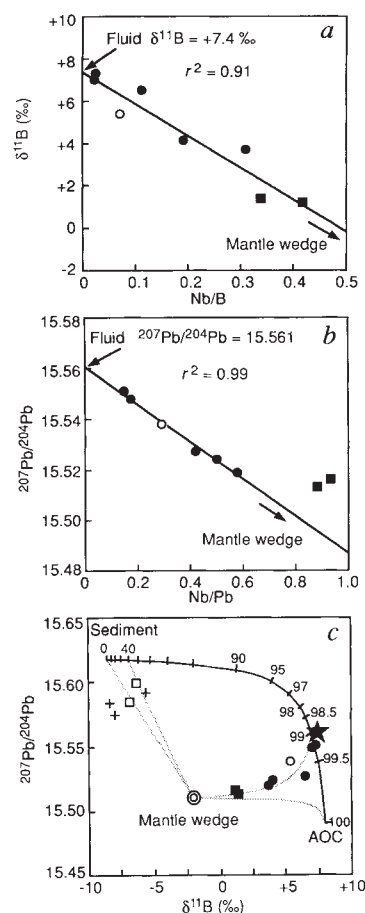
Concentrations and isotopic compositions of B and Pb were determined at the Pheasant Memorial Laboratory using the Cs₂BO₃-graphite-mannitol method²⁷ and the silica-gel method²⁸, respectively. Nb and major-element concentrations were measured at Rigaku Corporation by X-ray fluorescence spectrometry²⁹. B isotopic composition is expressed as per mil deviation ($\delta^{11}\text{B}$) relative to NIST SRM 951 boric acid thus; $\delta^{11}\text{B} = [(^{11}\text{B}/^{10}\text{B})_{\text{sample}} / (^{11}\text{B}/^{10}\text{B})_{\text{SRM951}} - 1] \times 10^3$. Replicate analyses of SRM 951 ($n=22$) and NIST SRM 981 Pb ($n=16$) gave mean values with standard deviations as follows: $^{11}\text{B}/^{10}\text{B} = 4.0527 \pm 0.0006$, $^{206}\text{Pb}/^{204}\text{Pb} = 16.941 \pm 0.003$, $^{207}\text{Pb}/^{204}\text{Pb} = 15.498 \pm 0.004$ and $^{208}\text{Pb}/^{204}\text{Pb} = 36.721 \pm 0.012$. Total procedural blanks for the period of analysis were 4 ng B and 150 pg Pb, which are negligible to the isotopic results.

* Values taken from refs 5, 24, 28, 30.

specific hydrous minerals such as amphibole and phlogopite^{14,15}, implying that the process of dehydration is not so simple or that there is some mixing or migration of fluid. On the other hand, continuous dehydration is consistent with observed successive B depletion during subduction-related progressive metamorphism^{16,17} and the estimated distribution of subduction-contaminated mantle beneath Japan, Korea and China^{18,19}.

The $\delta^{11}\text{B}$ of +7.4‰ estimated for the slab-derived fluid is consistent with the value for AOC (+2 to +9‰, with 20 p.p.m. B on average)^{6,7,16}, and clearly different from that of oceanic sediment after diagenesis (-12 to -6‰ with 120 p.p.m. B on average)^{5,20}. On the other hand, these Izu arc lavas have more radiogenic Pb isotopic compositions than MORB and AOC, approaching the values of pelagic sediment as observed in other arc volcanic rocks (for example, see Fig. 4 in ref. 21). Thus,

Fig. 3 a, b, Plots of Nb/B versus $\delta^{11}\text{B}$ (a) and Nb/Pb versus $^{207}\text{Pb}/^{204}\text{Pb}$ (b) of the Izu arc lavas. (The symbols are the same as Fig. 1.) These are equivalent to the plots of $1/\text{B}$ versus $\delta^{11}\text{B}$ and $1/\text{Pb}$ versus $^{207}\text{Pb}/^{204}\text{Pb}$ when we regard Nb as a constant. In such a diagram, two-component mixing is expressed as a single line. Thus linear relationships indicate the control of these values by mixing of two distinct components, slab-derived fluid and mantle wedge. The intercept of the regression line (solid line) with the y-axis represents the fluid composition (see text). r^2 is a correlation coefficient. Because of higher incompatibility of Nb than Pb, Nb/Pb of two rhyolites can be largely fractionated so that they are excluded from consideration. This type of plot may be performed for $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$, which yields fluid compositions of 18.392 ± 0.045 ($r^2=0.42$) and 38.320 ± 0.034 ($r^2=0.63$), respectively. c, Model calculation showing the mixing relationship between slab-derived fluid and mantle wedge. Solid curve with tick marks represents bulk mixing between typical AOC and pelagic sediment to form a homogeneous slab component. Shaded curves indicate mixing between different slab components and hypothetical MORB-like mantle wedge. The star symbol represents fluid composition for the Izu arc estimated from (a) and (b). High-Mg andesites from Setouchi (open squares) and Shimanto terrigenous shales (crosses) are also plotted. As B isotopic compositions of sediments change considerably during diagenesis (see ref. 5), the $\delta^{11}\text{B}$ of typical marine shale is used here instead of modern un lithified oceanic sediment. Note that even slight



addition of sediment to AOC dramatically raises the $^{207}\text{Pb}/^{204}\text{Pb}$ ratio of the slab component without lowering significantly $\delta^{11}\text{B}$, because sediment has much higher Pb/B than AOC. Compositions of assumed endmembers are: AOC^{6,7,9,16,32}, 20 p.p.m. B, $\delta^{11}\text{B} = +8.0$ ‰, 0.3 p.p.m. Pb and $^{207}\text{Pb}/^{204}\text{Pb} = 15.489$; sediment^{5,20,21}, 120 p.p.m. B, $\delta^{11}\text{B} = -8.5$ ‰, 36 p.p.m. Pb and $^{207}\text{Pb}/^{204}\text{Pb} = 15.617$; mantle wedge, Pb/B = 0.3, $\delta^{11}\text{B} = -2$ ‰ and $^{207}\text{Pb}/^{204}\text{Pb} = 15.510$.

both AOC and sediment are necessary to explain the fluid composition estimated here. However, because oceanic sediments have distinctly higher B contents and lower $\delta^{11}\text{B}$ compared to AOC, the amount of sediment in the subducting slab must be much smaller than that of AOC to maintain the higher $\delta^{11}\text{B}$ of the fluid. Bulk mixing calculation (Fig. 3c) shows that 99 wt% AOC with 1 wt% sediment fulfils the B and Pb isotopic compositions. The trends in our data are consistent with the global systematics in $^{10}\text{Be}/\text{Be}$ versus B/Be identified by Morris *et al.*², and provide further constraints on fluid sources. Without boron isotopes, Morris *et al.* were unable to distinguish between the possibilities of fluid coming from sediment alone, or fluid or melt coming from AOC, with a small sediment contribution. Our B and Pb isotopic data argue strongly in favour of the latter model. We conclude that AOC is the primary source of fluid in these arc systems, and any interpretation of trace elements and isotopic compositions of the arc lavas must take into account the incorporation of AOC-derived fluid.

We also measured B and Pb isotopic compositions of Miocene high-Mg andesites ('sanukitoids') from the Setouchi volcanic belt (Fig. 1). The sanukitoid is a primary andesite formed by partial melting of unusually hydrous mantle at relatively shallow level (pressure <15 kbar)²², and is regarded as a product of near-trench (fore-arc) volcanism resulting from the passive subduction of the Philippine Sea plate underneath the Eurasia plate which followed back-arc opening of the Sea of Japan²³. These rocks are highly enriched in both B and Pb, and show significantly lower $\delta^{11}\text{B}$ and higher $^{207}\text{Pb}/^{204}\text{Pb}$ compared to the Izu arc lavas (Table 1, Fig. 3c). This indicates that hydrous fluid from a source consisting of >40% sediment and <60% AOC has participated in the genesis of the sanukitoid magma. The present hypothesis is also supported by extraordinarily high Rb and K contents, and highly enriched Sr and Nd isotopic compositions of these rocks²⁴. It is conceivable that sediment in the subducting slab dehydrates at shallower depth than AOC. The striking contrast in B-Pb signature observed between Izu and Setouchi may reflect such a process. However, the isotopic compositions of H_2O -soluble elements in the slab are suggested to be largely homogenized by metamorphic process in the shallow subduction zone^{2,17,25}. If this is the case, the apparent proportion of sediment to AOC deduced from the fluid composition does not change regardless of the depth of the subducting slab. In fact, both B and Pb isotopic compositions of the fluid involved in the Izu arc are constant at depths of the WBZ between 150 and 220 km (Fig. 3a, b). In this case, the unusually high sediment/AOC ratio estimated for Setouchi can be explained by the participation of accreted sediment as a source of the fluid in addition to the subducted sediment and AOC. Actually, B and Pb isotopic compositions of these sanukitoids are indistinguishable from those of Miocene terrigenous shales (Fig. 3c) from the Shimanto accretionary prism²⁶, which parallels the Setouchi volcanic belt and the Nankai trough. In either case, fluid formation in the fore-arc region may be dominated by the dehydration of sediment. □

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A new carnivorous marsupial from the Palaeocene of Bolivia and the problem of marsupial monophyly

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THE distinctive soft anatomy and reproductive biology of marsupials sets them apart as a unique group within mammals. These features are, of course, absent in fossils, so it is difficult to determine marsupial origins and evolution: many of the proposed dental and skeletal characters are controversial¹ or primitive^{1,2}. A contribution of the alisphenoid bone to the tympanic bulla of the skull has long been considered a reliable diagnostic feature of the group^{1–5}. But this feature is lacking both in the borhyaenoid marsupial *Mayulestes ferox*, which I describe here, and the didelphoid *Pucadelphys andinus*^{6,7}, both from the early Palaeocene of Bolivia^{7–9}. Comparison with younger taxa suggests that this feature appeared several times independently within the group, and is thus an inappropriate defining character. What, then, is a marsupial?

Order Metatheria
Superfamily Borhyaenoidea
Family Mayulestidae nov.
Mayulestes ferox gen. et sp. nov.

Etymology: *Mayu*, river (Quechua) in reference to the alluvial plain in the Tiupampa area during the early Palaeocene, and *lestes*, predator; *ferox*, fierce.

Holotype: MNHC P 1249 (Museo de Historia Natural de Cochabamba, Bolivia), a partial skeleton including skull, mandible, 21 vertebrae and most limb bones.

Locality and horizon: Tiupampa (Department of Cochabamba), Bolivia. Santa Lucia Formation⁸. Age: Tiupampian (early Paleocene)^{9,13}.

Description: Very small size; I5/4, C1/1, P3/3, M4/4; no diastema; P¹ strongly and P₁ slightly set obliquely in jaw; P3 much higher than M1; protocone of M^{1–3} more robust than in *Allqokirus*⁶, paracone smaller than metacone, straight centro-

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crista, small paracingulum, no metacingulum, stylar shelf medium-large, small anteriorly projecting cusp A, cusp B largest and linked to paracone by a distinct preparacrista, no cusp C, cusp D smaller than B with a small crista directed toward metacone, large postmetacrista, M^2 and M^3 wider than long, L/W ratio smaller than in *Allqokirus*, ectoflexus deeper than in *Allqokirus*; lower molars smaller and narrower than in *Allqokirus*, slightly increasing in size from M_1 to M_3 ; trigonid much higher and wider than talonid; strong anterolabial and posterolabial cingulids; trigonid little opened lingually; metaconid and paraconid subequal in size; talonid distinctly basined; large hypoconid subequal in size with lingually placed hypoconulid; entoconid small; short rostrum; palate without vacuities; pterygoid with broad ventral lamina and conspicuous

hamulus; nasals expanded posteriorly with a W-shaped frontal suture and contacting the lacrimals; no transverse canal; conspicuous medial process of the squamosal; no tympanic process of the alisphenoid; shallow alisphenoid hypotympanic sinus with a large contribution of the squamosal and confluent posteriorly with the epitympanic recess; no prootic canal; promontorium with a deep sulcus for internal carotid artery; pars mastoidea contributes to the occipital shield.

So far, the two oldest skulls of undisputed marsupials are those of *Pucadelphys andinus*^{6,7} and *Mayulestes ferox* (Fig. 1) from the earliest Palaeocene of Bolivia^{7,9}. Recently Deltatheroidea (a predominantly Old World group of primitive tribosphenic mammals) have been included in the Metatheria⁵ on the presence of a tympanic process of the alisphenoid and a postcanine dental formula of 3P and 4 to 3M; the borhyaenoids have been regarded as close relatives of the deltatheroidans¹⁴. However, the skulls of *Pucadelphys* and *Mayulestes* do not have an alisphenoid process^{6,7} (Figs 1b and 2a). As suggested in Figs 2 and 3, the borhyaenoids are characterized by the appearance of a tympanic process of the alisphenoid in the *Sipalocyon-Cladosictis* clade. Other borhyaenoids (*Sallacyon* (Fig. 2b), *Borhyaena*, *Prothylacynus*, *Paraborhyaena*, *Lycopsis*¹⁵ and *Thylacosmilus*) lack an alisphenoid process (Fig. 3). Consequently, contrary to earlier statements^{7,14}, this absence probably represents the plesiomorphic condition. Therefore, the absence of an alisphenoid process in the oldest known didelphoid skull (*Pucadelphys andinus*), contrary to what is observed in other didelphoids, favours the hypothesis that the structure is acquired in the other taxa of the superfamily rather than lost in *Pucadelphys*^{7,14}.

Thus, it is suggested that a tympanic process of the alisphenoid appeared several times independently during metatherian evolution and consequently, that feature, commonly regarded as a synapomorphy of marsupials^{1,2,5,14}, should not be used to diagnose this group. Other metatherian synapomorphies observable in fossils include the number of premolars reduced to three (the presence of four molars is a symplesiomorphy¹) and the twinning of entoconid and hypoconulid. However, the dental formula of the deltatheroidans (P3/3 and M3-4/4) has been regarded as convergent with that of the marsupials¹⁶ and twinned entoconid and hypoconulid are also found in some placentals (some Chiroptera¹, Cynocephalidae, Tupaiidae¹⁷, Talpidae and Soricidae¹⁸), although they are commonly regarded as convergent to marsupials^{1,19}. Marsupials are also diagnosed by the loss of the stapedial artery in adults²⁰⁻²², by a single tooth replacement (M1 or DP3, according to interpretation) and by important specializations of the reproductive organs and physiology^{1,2}. However, these features are very difficult to observe (the first two) or not observable (the third) in fossils. Furthermore, the stylar cusp C, regarded as a possible marsupial synapomorphy^{1,2}, is absent in *Mayulestes* (Fig. 1) and *Allqokirus*⁷, and either absent or very reduced in *Protalaphodon*²³ and *Anchistodelphys*²⁴.

The presence of a reduced prootic canal with intramural opening has also been regarded as a marsupial synapomorphy²²; however, that feature is present only in didelphoids, caenolestoids, some dasyurids and some late Cretaceous taxa from North America²²; it is absent in all other marsupials. The participation of the jugal to the glenoid fossa, cited as a metatherian synapomorphy^{4,14}, is a symplesiomorphy^{2,15} also found in triconodontids²⁵, morganucodontids²⁶ and *Vincelestes*²⁷. The dental synapomorphies mentioned elsewhere¹⁴ to diagnose the Marsupialia are carnivorous specializations which are found in at least six major groups (Deltatheroidea, Stagodontidae, Borhyaenoidea, Thylacinidae, Creodonta and Carnivora). For this reason they cannot be shown to represent unique features and they are unreliable synapomorphies. The only, apparently unique, synapomorphy of marsupial skeleton is the occurrence of a groove for the prootic sinus located on the lateral side of the petrotic and covered by the squamosal (in one case possibly

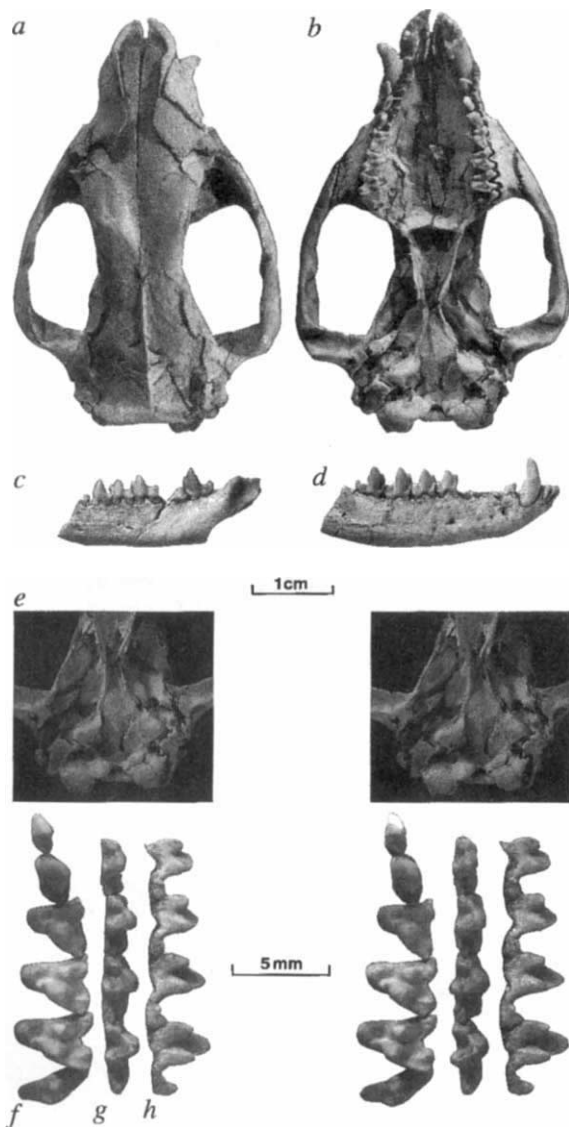


FIG. 1 *Mayulestes ferox*, gen. et. sp. nov. (holotype, MHNC 1249): a, skull in dorsal view; b, skull in ventral view; c, left lower jaw in lateral view; d, right lower jaw in lateral view. e, stereopair of the basicranium; f, stereopair of left upper jugal teeth in occlusal view; g, stereopair of right lower molars in occlusal view; h, stereopairs of right lower molars in medial view. c, d, h Show the incisor formula I5/4, the slight increase in size from M_1 to M_3 and the M_3 subequal in size to M_4 , which contradicts the borhyaenoid synapomorphies (incisors reduced to I4/3 and rapid increase in size from M_1 to M_4 and from M^1 to M^3) proposed in earlier work^{4,14}.

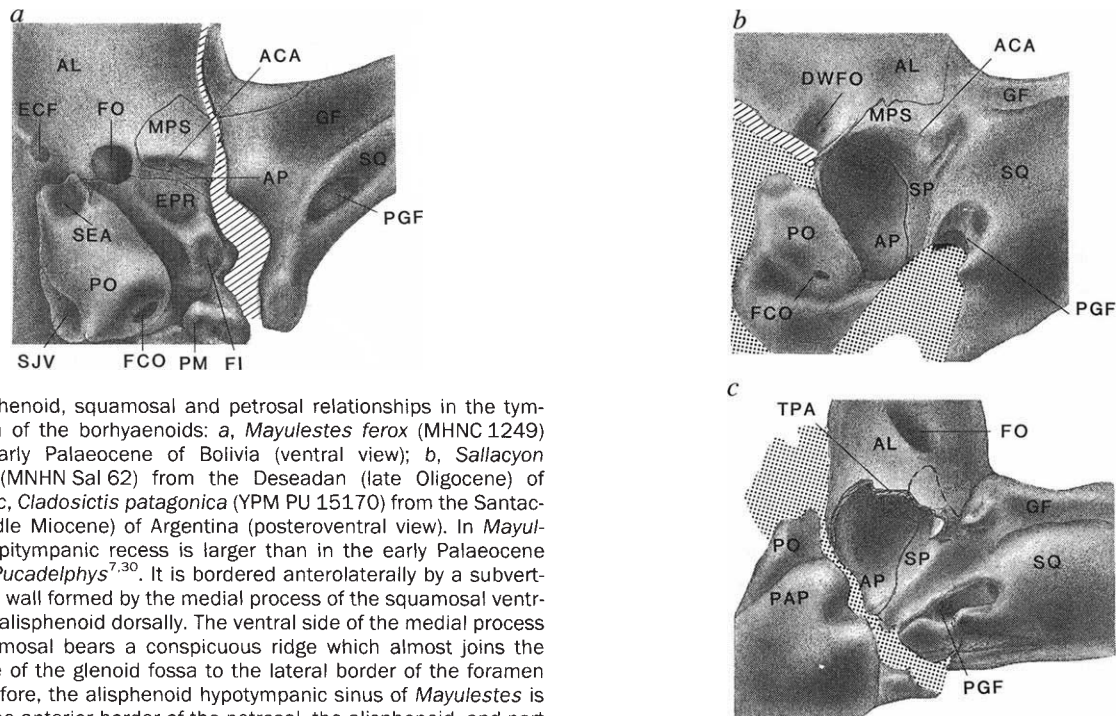
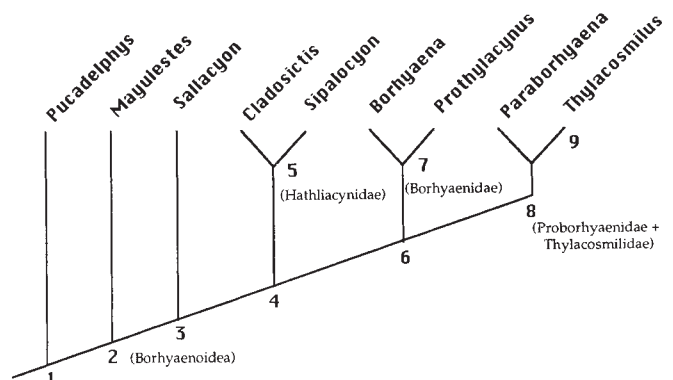


FIG. 2 Alisphenoid, squamosal and petrosal relationships in the tympanic region of the borhyaenoids: *a*, *Mayulestes ferox* (MHNC 1249) from the early Palaeocene of Bolivia (ventral view); *b*, *Sallacyon hoffstetteri* (MNHN Sal 62) from the Deseadan (late Oligocene) of Bolivia^{28,29}; *c*, *Cladosictis patagonica* (YPM PU 15170) from the Santacrucian (middle Miocene) of Argentina (posteroventral view). In *Mayulestes* the epitympanic recess is larger than in the early Palaeocene didelphoid *Pucadelphys*^{7,30}. It is bordered anterolaterally by a subvertical concave wall formed by the medial process of the squamosal ventrally and the alisphenoid dorsally. The ventral side of the medial process of the squamosal bears a conspicuous ridge which almost joins the medial edge of the glenoid fossa to the lateral border of the foramen ovale. Therefore, the alisphenoid hypotympanic sinus of *Mayulestes* is formed by the anterior border of the petrosal, the alisphenoid, and part of the medial process of the squamosal. In *Sallacyon hoffstetteri*, the relatively large epitympanic recess is located posteromedial to a deep alisphenoid sinus. That sinus is made of the alisphenoid posteromediodorsally and the medial process of the squamosal anterolateroventrally. The dorsal side of the process bears a strong ridge which almost joins the medial edge of the postglenoid process to the lateral edge of the foramen ovale. In *Cladosictis patagonica* (YPM PU 15170 and 15046) and *Sipalocyon gracilis* (YPM PU 15154) the portion of the alisphenoid anterior to the medial process of the squamosal extends ventrally and posteriorly and forms a wing which floors the tympanic cavity. In these genera the medial process of the squamosal is internal to the alisphenoid sinus (dotted line in Fig. 3c). In *Pucadelphys andinus*, an early Palaeocene didelphoid from Tiupampa, there is no alisphenoid hypotympanic sinus and the epitympanic recess is a small depression

in the periotic, anterolateral to the ventral opening of the facial canal, anterior to the fossa incudis and is partially underlain by a large medial process of the squamosal^{6,7,30}. It could represent the primitive condition for marsupials. ACA, Anterior crest of the alisphenoid hypotympanic sinus; AL, alisphenoid; AP, alisphenoid portion of the alisphenoid hypotympanic sinus; DWFO, dorsal wall of the foramen ovale; ECF, entocarotid foramen; EPR, epitympanic recess; FCO, fenestra cochleae; FO, foramen ovale; FI, fossa incudis; GF, glenoid fossa; MPS, medial process of the squamosal; PAP, paroccipital process; PM, pars mastoidea; PGF, postglenoid foramen; PO, promontorium; SEA, sulcus for the entocarotid artery; SJV, sulcus for the internal jugular vein; SP, squamosal portion of the alisphenoid hypotympanic sinus; SQ, squamosal; TPA, tympanic process of the alisphenoid. Hatching indicates breaks; dots represent matrix or plaster. Illustrations not drawn to scale.

FIG. 3 Phylogenetic relationships of major groups of borhyaenoids (known by skulls with basicranium) and *Pucadelphys andinus* (outgroup) using features not related to dental morphology (the skull of *Lycopsis*¹⁵ was not available during this study, and has not been considered). Node 1: presence of a medial process of the squamosal almost reaching the foramen ovale. Node 2: Borhyaenoidea. Alisphenoid hypotympanic sinus formed by the squamosal, the alisphenoid, and the epitympanic recess; loss of the epipubic bones (unknown for *Sallacyon* and *Mayulestes*); loss of prootic canal (loss is also found in dasyuroids²²) (the presence of a prootic canal is a plesiomorphy among marsupials³¹). Node 3: pars mastoidea of the petrosal internal; loss of participation of the alisphenoid in the glenoid fossa; number of incisors reduced to 4/3; strong reduction of the hamular processes and laminae of the pterygoids and formation of two crests that connect (without level difference) the posterior edge of the palate to the basicranium. Node 4: reduction of the fossa subarcuata; foramen ovale totally enclosed by the alisphenoid and floored by a thick medial expansion of that bone; large mastoid process formed by the paroccipital process and the posttympanic process. Node 5: Hathliacynidae. Snout elongated with diastema between premolars and between P1 and canine; development of a tympanic process of the alisphenoid which contacts the paroccipital process and totally floors the tympanic cavity ventrally. Node 6: development of the alisphenoid hypotympanic sinus which tends to enter the skull dorsally; very large and robust animals which tend to have an ankylosed symphysis. Node 7: Borhyaenidae. Large exposure of the squamosals on the posterolateral face of the occipital shield. Node 8: Proborhyaenidae + Thylacosmilidae. Upper canines with open pulp cavity⁴. Node 9: Acquisition of a tympanic bulla formed by an anterior expansion of the paroccipital process of the exoccipital and the posttympanic process of the squamosal which contact the alisphenoid and the squamosal anteriorly.



by the alisphenoid)²². Moreover, the dental formula and the twinned entoconid and hypoconulid (present in all known marsupials) are perhaps the least ambiguous other synapomorphies that could diagnose marsupials, both fossil and recent, although we know that they are (respectively, possibly and certainly) homoplastic. □

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A stoichiometric analysis of the zooplankton–phytoplankton interaction in marine and freshwater ecosystems

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IN the 35 years since A. C. Redfield's classic paper¹, the use of elemental ratios has become widespread in marine and freshwater phytoplankton studies^{2,3}. But nutrient ratios have only recently been studied elsewhere in pelagic ecosystems, such as the producer–consumer interface^{4,5}. Here we report the results of the first study, to our knowledge, of N:P ratios in pelagic producers and consumers (phytoplankton and zooplankton) in lacustrine and marine habitats. The N:P ratio of phytoplankton was higher in lakes than in marine sites; however, N:P ratios were higher in marine zooplankton than in freshwater zooplankton. The elemental imbalance of the phytoplankton–zooplankton interaction ($N:P_{\text{food}} - N:P_{\text{consumers}}$) in lakes was positive and exceeded the negative imbalance in marine sites; thus P-deficient food may limit zooplankton growth in lakes but not in oceans. Stoichiometric calculations⁶ indicated that consumer-driven nutrient recycling ratios in lakes may be 4–6 times higher than in marine systems. Consistent with this difference, phytoplankton P-limitation was more prevalent in lakes than in marine sites. Thus, the ecological stoichiometry of the zooplankton–phytoplankton interaction differs qualitatively in freshwater and marine ecosystems.

During the summers of 1992 and 1993, we sampled lakes of varying size, trophic state and food web structure in northern Wisconsin and Michigan (USA) and northwestern Ontario (Canada) as well as nearshore and offshore marine sites in the Atlantic and Pacific oceans (Table 1). At each site, we measured N:P ratios (N:P) in suspended particulate material that passed through an 83 µm mesh (seston, assumed to be dominated by phytoplankton) and in zooplankton (collected with a 153-µm mesh tow net) in the surface layers. We compared zooplankton N:P and phytoplankton N:P to determine if the nutrient stoichiometry of the producer–consumer interaction differs between

these ecosystem types, and thus, whether zooplankton in lakes and oceans face different food quality constraints and differ qualitatively in their N and P recycling. We also assessed N and P limitation of phytoplankton growth to determine if nutrient limitation patterns reflect the potential differential effects of zooplankton on N and P availability in lakes and oceans.

Freshwater and marine sites differed in the N:P of both seston and zooplankton. Lake seston had high N:P characteristic of P-limited growth⁷ whereas marine seston values were significantly lower ($P < 0.0001$; Fig. 1a) and similar to the Redfield ratio (16:1 by atoms¹). Unlike seston N:P, marine zooplankton N:P exceeded the N:P of freshwater zooplankton ($P = 0.054$; Fig. 1b). Although little data exist regarding species-specific N:P for marine or freshwater taxa, this difference in zooplankton N:P is consistent with what little is known about zooplankton N:P ratios. Marine zooplankton N:P encompassed values previously reported for various copepod species^{8,9}; freshwater N:P approached the low N:P of various cosmopolitan cladoceran taxa⁸, especially *Daphnia* (atomic N:P of 12–15⁸), a conspicuous member of the zooplankton in most of the study lakes. Differences in zooplankton size structure may also have contributed to the difference in zooplankton N:P in lakes and oceans. We measured zooplankton N:P in four size classes between 153 µm and 1,050 µm for a small subset of our sites. For both lacustrine and marine sites, large size-classes had lower N:P than small size-classes. Thus, zooplankton from lakes may have been more strongly skewed towards large animals than the marine zooplankton.

We characterized the stoichiometry of the phytoplankton–zooplankton interaction by calculating the elemental imbalance between producers and consumers for each site ($N:P_i = N:P_{\text{seston}} - N:P_{\text{consumers}}$). In lakes, $N:P_i$ was positive and substantially exceeded the negative $N:P_i$ of marine sites ($P < 0.0001$; Fig. 1c). Seston N:P exceeded zooplankton N:P for 29 of the 30 lake observations, but for only 4 of the 14 marine samples. The elemental imbalances between producer and consumer demonstrated here apply only to the larger metazoan zooplankton captured with the 153-µm net. In many ecosystems, especially oligotrophic oceanic areas²², microconsumers are important grazers and might therefore contribute to the particulate matter collected in our seston fraction, especially in offshore marine sites. This potential contribution is, however, unlikely to alter the habitat difference in producer–consumer elemental imbalance as, based on our size fractionation studies, microconsumers are

TABLE 1 Sampling site summary

Lakes	No. of sites	TP (μM)*	TN (μM)†	Chl ($\mu\text{g l}^{-1}$)‡
Northern Lakes Long-Term Ecological Research Site (northern Wisconsin, USA)	18	0.15–0.63	13.4–65	2.35–58.94
University of Notre Dame Environmental Research Center (Upper Peninsula, Michigan, USA)	9	0.25–0.79	12.1–46.8	3.89–17.25
Experimental Lakes Area (northwestern Ontario, Canada)	9	0.057–1.06	15.9–83.8	0.57–22.4
Marine and Estuarine sites				
North Inlet Long-Term Ecological Research Site (South Carolina, USA)	3	0.37–0.92	37.5–56.0	22.5–43.8
Long Island Sound (Stony Brook Harbor, Flax Pond; New York, USA)	2	2.04–2.16	41.9–46.6	3.01–3.91
Damariscotta River Estuary (Maine, USA)	2	1.01–1.10	33.7–33.9	—
Gulf Stream, western Atlantic Ocean (4–40 km off Melbourne; Florida, USA)	4	0.18–0.43	35.0–41.9	0.71–1.01
Gulf of California (5–10 km off Puerto Penasco; Sonora, Mexico)	3	1.11–1.24	36.0–29.6	5.90–10.8
Southeast Pacific Ocean (26°31'277"–27°12'80"N/123°1'120"–121°4'117" W)	3	0.49–0.58	37.3–39.4	1.00–1.16

Locations, number of sites sampled in the vicinity of each location, limits of nutrient concentrations (TP, total P; TN, total N), and limits of phytoplankton biomass (as indicated by chlorophyll concentration) are given.

* TP is the sum of total dissolved P, determined colorimetrically after ultraviolet oxidation²⁰, and particulate P, determined by colorimetric analysis of P after persulphate oxidation²⁴ of material filtered onto precombusted glass-fibre filters (0.7 μm porosity).

† TN is the sum of total dissolved N, determined colorimetrically after ultraviolet oxidation²⁰, and particulate N, determined by elemental analysis of material filtered onto precombusted glass-fibre filters (0.7 μm porosity).

‡ Chlorophyll concentrations were determined fluorometrically²¹ using methanol extracts of material filtered on glass-fibre filters (0.7 μm porosity).

likely to have high N:P ratios. The potential disproportionate contribution of microconsumers to marine seston values would therefore only imply that actual marine imbalances are even more strongly negative than we measured.

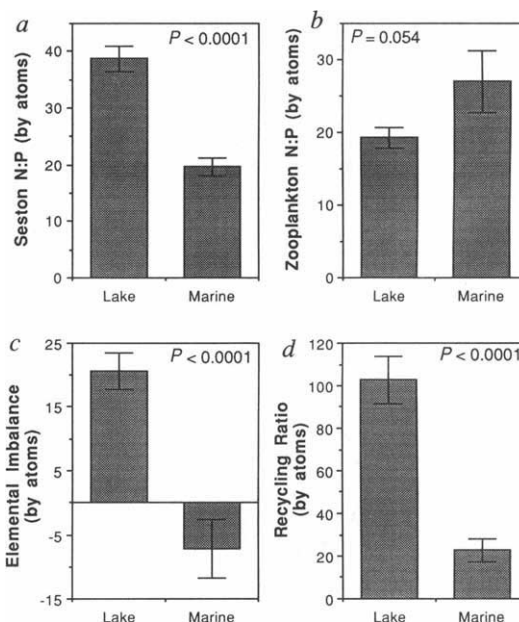
Strong differences in elemental ratios between producers and consumers affect both consumer individuals and the ecosystem. For individuals, mineral (especially P) limitation of zooplankton growth occurs when food has insufficient nutrient (P) content^{10–12}, although this possibility is controversial^{13–15}. Our results indicate that elementally based food quality constraints are stronger in fresh water where P-limited phytoplankton growth results in food potentially deficient in P, especially for taxa like *Daphnia* with high P demands⁸. We compared seston

C:P ratios in our study lakes to threshold values^{10–12} indicative of P-limitation of growth of *Bosmina* and *Daphnia* (common freshwater zooplankters; Fig. 2). C:P ratios exceeded even the most conservative thresholds in the majority of our study lakes. Thus, P-limitation of zooplankton growth may be ubiquitous in lakes. We know of no studies establishing P-limitation thresholds for marine zooplankton species; however, marine seston C:P was considerably lower than C:P in the lakes sampled (Fig. 2) and thus P-based food quality constraints appear unlikely. The weaker elemental imbalance in marine sites (Fig. 1c) also indicates that food quality constraints based on elemental content are unlikely for marine zooplankton.

At the ecosystem level, consumer–producer differences in N:P

FIG. 1 N:P ratios (by atoms) characterizing the zooplankton–phytoplankton interaction in lakes and marine sites. a, N:P ratio of suspended particulate matter (seston). b, N:P ratio of zooplankton. c, Elemental imbalance ($N:P_i = N:P_{\text{seston}} - N:P_{\text{consumers}}$) between phytoplankton and zooplankton. d, Estimated N:P ratio of zooplankton-recycled nutrients calculated using stoichiometric theory⁶ ($N:P_{\text{recycled}} = N:P_{\text{seston}}(1-L) - LN:P_{\text{consumers}}/(1-L)$ when $N:P_i > 0$; $N:P_{\text{recycled}} = N:P_{\text{seston}}(1-L)/(1-LN:P_{\text{consumers}}/N:P_i)$ when $N:P_i \leq 0$. L, Maximum accumulation efficiency). In this case, L was set at 0.75, a somewhat conservative value given data indicating that zooplankton feeding on P-deficient food reduce P-release to values approaching zero (that is, $L \approx 1.0$)¹². Error bars indicate ± 1 s.e. Data were first examined for normality and then log-transformed when necessary to stabilize the variance (seston N:P, zooplankton N:P, recycling N:P). For all variables except $N:P_i$, variances were uniform for the two groups. Means for lake and marine sampling sites were compared using a standard t-test or a t-test assuming unequal variances ($N:P_i$ only). P values given reflect the results of these t-tests.

METHODS. Lake and marine seston values from each study site used in calculating these overall means were the mean of duplicate collections from the surface mixed layer at each sampling station. Particulate matter (pre-screened through 83- μm mesh to remove most larger zooplankton) was collected onto pre-combusted (24 h at 450 °C) glass-fibre filters (0.7 μm porosity) and analysed for P content by colorimetric means after persulphate oxidation²⁰, or for C and N using a Perkin-Elmer Model 2400 CHN analyser. Zooplankton data represent means from duplicate vertical tows made with a metered tow net constructed of 153- μm mesh. In lakes and shallow marine sites, tows were made from 0.5 m above the bottom to the surface. For deeper marine sites, tows encompassing the entire surface mixed layer were taken. When zooplankton samples contained appreciable densities of large phytoplankton cells, zooplankton were separated from phytoplankton by narcotization and settling. Known aliquots of zooplankton were placed on



precombusted, preweighed glass fibre filters. Filters with animals were dried (60 °C), reweighed, and analysed for P or N with the same methods used for seston. N:P ratio for zooplankton was then calculated for each duplicate net tow based on %P and %N per unit dry weight. The mean of these duplicates for each sampling site was used in calculating the overall means shown.

can affect the relative rates of nutrient recycling by zooplankton⁵. Metazoan zooplankton regulate their N and P content at fixed ratios^{8,12}. Thus, when consuming food with an N:P higher than their body N:P, animals will retain P and differentially recycle N. The converse occurs when animal N:P exceeds food N:P. Reflecting the mass balance of these processes, the N:P of potential zooplankton recycling can be calculated from food N:P and zooplankton N:P⁶. Recycling N:P was significantly ($P < 0.0001$) and substantially (4–6 times) higher for lakes than for marine sites (Fig. 1d). High N:P recycling ratios in lakes were reflected in assays of the relative degree of N and P limitation of phytoplankton (measurements of N and P uptake in the dark⁷): lake phytoplankton were significantly more P-deficient (relative to N) than marine phytoplankton (Mann-Whitney U-test, $P < 0.05$). This association of recycling N:P and phytoplankton nutritional status raises the issue of cause and effect. Whole-ecosystem and enclosure experiments in lakes have shown that shifts in the zooplankton community can change the element (N versus P) primarily limiting phytoplankton growth¹⁶. The correspondence between relative N versus P limitation and the stoichiometry of the zooplankton-phytoplankton interaction in marine and freshwater ecosystems is consistent with the suggestion that zooplankton recycling can qualitatively alter the relative availability of N and P. However, our results do not demonstrate that differences in phytoplankton nutrient limitation were caused by differential nutrient recycling but do indicate that the effect of zooplankton on N and P availability differed qualitatively in the marine and freshwater habitats sampled. In lakes, zooplankton nutrient recycling further accentuates P limitation of phytoplankton whereas in marine systems, zooplankton recycling of N and P is less disproportionate but may amplify N limitation.

Although Redfield's stoichiometric approach has provided insights into the coupling of biogeochemical cycles in the sea¹, ecological stoichiometry is still in its infancy¹⁷. However, increasing data demonstrate that studies of multiple elements can provide new insights into the processes affecting behavioural patterns, population regulation, and community interactions in

a variety of study organisms, from mammals¹⁸ to microorganisms¹⁹. In our study, analyses of the ratios of biologically important elements (C, N, P) in functionally dominant food web components lead us to conclude that the producer-consumer interaction differs qualitatively in marine and freshwater pelagic ecosystems. This difference probably results in contrasting dynamics of food quality and nutrient recycling at the zooplankton-phytoplankton interface in lakes and oceans. □

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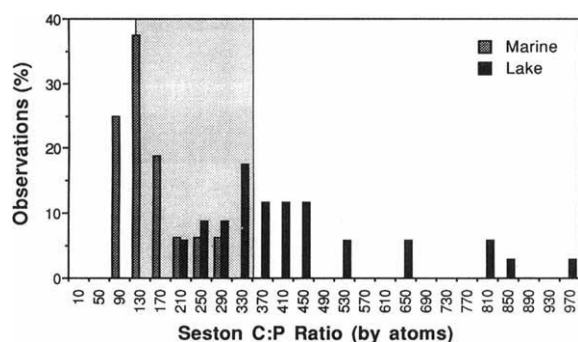


FIG. 2 Relative frequency of seston C:P ratios (by atoms) indicative of P-based food quality for zooplankton in lakes and marine sites. The boxed area indicates the range of values of recently published^{10–12} food C:P ratios indicative of a transition from C (energy)-limitation of zooplankton growth to limitation by P-content for two common freshwater zooplankton taxa (*Bosmina* sp. and *Daphnia* sp.). Values higher than this range indicate a strong potential for the occurrence of P-based food quality constraints on growth of these species. The majority of lakes sampled had seston C:P values higher than this range. High C:P ratios potentially reflect contributions of allochthonous detritus (high in C relative to P) rather than phytoplankton composition; we evaluated this possibility by calculating the C concentration of phytoplankton from directly measured chlorophyll concentrations using a C:chl ratio characteristic of P-limited phytoplankton (200:1 by mass⁷). These calculations indicated that chlorophyll-containing particles accounted for an average of 90% of the particulate C collected on filters. This indicates that allochthonous organic matter did not substantially interfere with our estimates of phytoplankton C:P.

The asexual ploidy cycle and the origin of sex

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SEX involves syngamy (gamete fusion), which doubles the amount of DNA in a cell, and meiosis¹, which halves it. The result is a 'ploidy cycle' of alternating diploid and haploid phases. Asexual reproduction does not require changes of ploidy, and yet asexual forms may have ploidy cycles. Here I show that such cycles lessen the mutation load, compared with permanent diploidy or polyploidy, and are thus likely to evolve in cases where it is always advantageous to have more than one copy of the genome per cell. The asexual ploidy cycle could have facilitated the origin of sex, by providing a means of orderly genetic reduction available immediately after the origin of syngamy.

The ploidy of some asexual forms may increase by endomitosis (replication not followed by cell division) and decrease by reduction (cell division not preceded by replication). This can cause variation of either the number of copies of the genome per nucleus (a ploidy cycle *sensu stricto*), of the number of nuclei per cell (a karyonic cycle), or both. Strict alternation of endomitosis and reduction leads to a haplo-diploid cycle, while successive endomitoses followed by reductions lead to complex cycles with polyploid phases. Mitoses may occur at some or all levels of ploidy^{2–4}.

It is unclear why asexual ploidy cycles have evolved. This can hardly happen if haploidy is always favoured⁵. The necessity of a back-up copy of DNA for repair⁶ might explain permanent diploidy, but not the ploidy cycle. The environment may directly favour different levels of ploidy at different times⁷⁻⁹, but no data currently support this. Here I show that the asexual ploidy cycle may be a mutation load-reducing mechanism, when diploidy or polyploidy is always favoured. Some unicellular polygenomic phagotrophs, both asexual and sexual, are so large that a single copy of the genome would be certainly insufficient^{2,10}, whereas in other cases the advantage of polyploidy is not obvious.

Consider first asexual N -ploids without the ploidy cycle. The succession of events during a generation is mutation, asexual reproduction, selection. If mutations are independent, rare and always deleterious, a mutation-free offspring can be produced, with the probability e^{-NU} , only by a mutation-free parent, where U is the mutation rate per haploid genome. At equilibrium the decline of the frequency of the mutation-free genotype after mutation must be equal to its increase after selection. Thus, the fitness of this genotype must exceed the mean population fitness by e^{NU} , and the mutation load L is always $1 - e^{-NU}$ (refs 11-13).

Assume now that between mutation and asexual reproduction an individual may undergo, with probability H , the asexual ploidy cycle. In its course all N genomes except a randomly chosen one are destroyed, after which N -ploidy is immediately restored, making all genomes of an individual identical. With $H=1$, $L=1 - e^{-U}$ (ref. 11), because an obligate ploidy cycle effectively leads to haploidy, whereas with $H<1$ the load can be larger.

If all mutations are equally deleterious, an individual can be described by two numbers: k , the number of unique mutations that appeared after the last ploidy cycle in the lineage and are present in only one genome, and m , the number of mutations common to all N genomes. Having a common mutation is an irreversible condition. Thus, to calculate the mutation load it is sufficient to consider only the individuals with $m=0$ and to find the flow from them to those with $m>0$, by analogy with the argument for the case without the ploidy cycle. Let us find how frequently an individual with $k \geq 0$ and $m=0$ before mutation produces an offspring with K unique and M common mutations. If the ploidy cycle did not occur, $M=0$, and $K \geq k$ with probability $e^{-NU}(NU)^{K-k}/(K-k)!$ (ref. 11). Otherwise, $K=0$, whereas $M=0$ with probability $(1-1/N)^k e^{-U}$ (the genome surviving the ploidy cycle does not carry a pre-existing mutation with the probability $1-1/N$, and k such mutations are distributed independently; the genome receives no fresh mutations with the probability e^{-U}). Thus, $x'(i)$ and $x(i)$, the absolute numbers of individuals with no common and i unique mutations after reproduction and before mutation, respectively, are connected by:

$$x'(i) = \sum_{j=0}^i A_{i,j} x(j),$$

$$A_{i,j} = \begin{cases} (1-H)e^{-NU} + He^{-U}; & i=j=0 \\ H(1-1/N)^j e^{-U}; & i=0 \text{ and } j>0 \\ (1-H)e^{-NU}(NU)^{i-j}/(i-j)!; & i>0 \text{ and } j \leq i \\ 0; & \text{other } i \text{ and } j \end{cases} \quad (1)$$

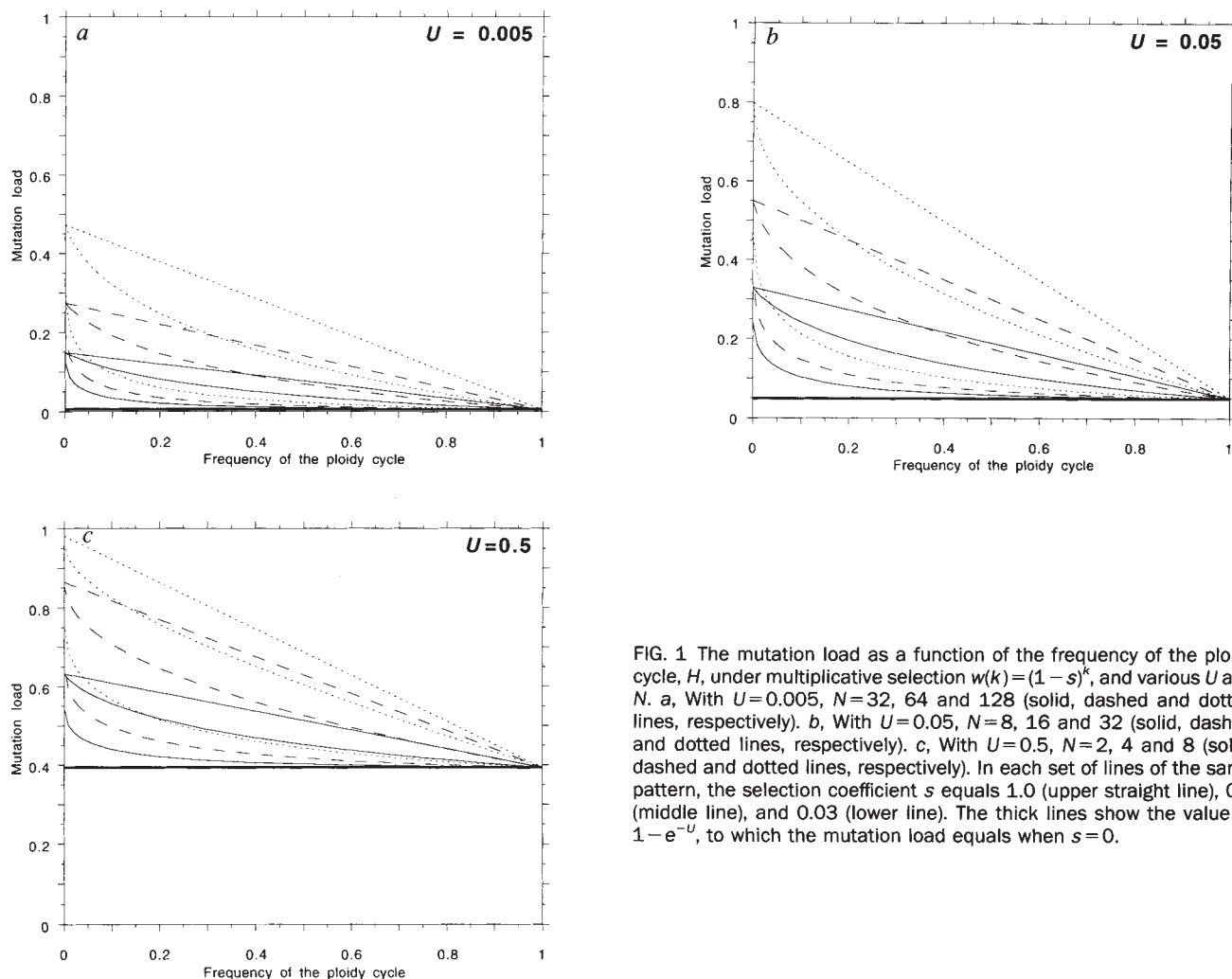


FIG. 1 The mutation load as a function of the frequency of the ploidy cycle, H , under multiplicative selection $w(k) = (1-s)^k$, and various U and N . a, With $U = 0.005$, $N = 32, 64$ and 128 (solid, dashed and dotted lines, respectively). b, With $U = 0.05$, $N = 8, 16$ and 32 (solid, dashed and dotted lines, respectively). c, With $U = 0.5$, $N = 2, 4$ and 8 (solid, dashed and dotted lines, respectively). In each set of lines of the same pattern, the selection coefficient s equals 1.0 (upper straight line), 0.3 (middle line), and 0.03 (lower line). The thick lines show the value of $1 - e^{-U}$, to which the mutation load equals when $s = 0$.

To obtain the equations connecting $x(i)$ in the successive generations, we need to incorporate selection, multiplying the j th column of $A = (A_{ij})$ by $W_{\max}w(j)$, where $W_{\max}$ is the absolute fitness of the mutation-free genotype and $w(j)$ is the fitness of individuals with no common and j unique mutations relative to $W_{\max}$. At equilibrium, where the frequencies of all genotypes are constant, $W_{\max}\lambda = \bar{W}$, where λ is the leading eigenvalue of $A_w = (A_{ij}w(j))$ and $\bar{W}$ is the mean population fitness. Thus, $L = 1 - \lambda$.

Two limit cases can be considered analytically. Under the harshest possible selection ($w(j) = 0$ with $j > 0$: only mutation-free individuals survive), $\lambda = A_{0,0}$ and $L = 1 - (He^{-U} + (1-H)e^{-UN})$. In contrast, under the weakest possible selection ($w(j) \equiv 1$: selection against unique mutations can be ignored because mutations are removed only when they become common after a ploidy cycle) the load is the same as with permanent haploidy. According to the Perron-Frobenius theorem, $\lim_{g \rightarrow \infty} \log A_{0,0}^g = \log \lambda$, where $A_{0,0}^g$ is the probability that after g generations the descendant of a mutation-free ancestor remains mutation-free. During these g generations there were no ploidy cycles with probability $(1-H)^g$, otherwise the last ploidy cycle occurred b generations before the descendant ($0 \leq b < g$) with probability $H(1-H)^b$. In these cases the descendant is mutation-free with the probabilities e^{-gNU} and $e^{-gU - b(N-1)U}$, respectively. Thus,

$$A_{0,0}^g = e^{-gU} H \sum_{b=0}^{g-1} (1-H)^b e^{-b(N-1)U} + (1-H)^g e^{-gNU} \quad (2)$$

and $\lim_{g \rightarrow \infty} \log A_{0,0}^g = -U$, so that $L = 1 - e^{-U}$ with any N , U and $H > 0$.

Under any $w(j)$, $L = 1 - e^{-NU}$ if $H = 0$ and $L = 1 - e^{-U}$ if $H = 1$. With intermediate $w(j)$ and H , the load can be found by evaluating λ numerically. Figure 1 shows the data under multiplicative selection. The results with other selection regimes are similar, so that here epistasis¹¹⁻¹⁴ is not essential. The load always decreases with increasing H . Under strong selection this decrease is close to linear, whereas under a more plausible¹⁵ weak selection even rare ploidy cycles decrease the load greatly.

Thus, the asexual ploidy cycle generates variability in the total number of mutations per individual by either removing unique mutations or converting them into common mutations. This decreases the mutation load, as well as the variance in the number of unique mutations per individual (Fig. 2), compared with permanent diploidy or polyploidy. With other successions of the

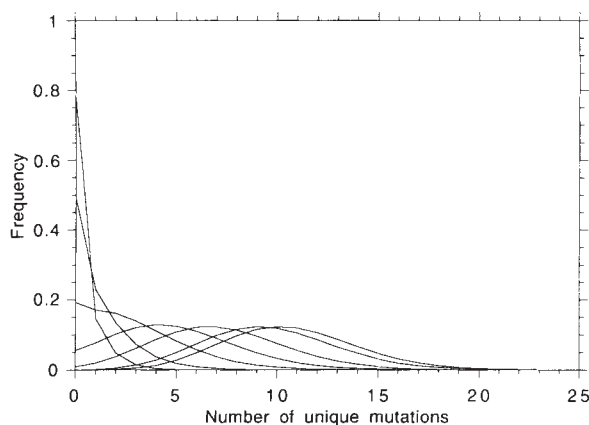


FIG. 2 Equilibrium distributions of the number of unique mutations in individuals with no common mutations under $U = 0.005$, $N = 64$, $s = 0.03$ and $H = 0.0, 0.00001, 0.001, 0.01, 0.05, 0.2, 0.5$ (the means decrease when H grows). With $H = 0.0$ the distribution is Poisson with the parameter $UN/s = 10.667$, whereas with $H = 1.0$ all individuals have no unique mutations.

events during a generation the results are analogous. Because only asexuals are considered, the load arguments are sufficient for evolutionary conclusions to be made^{13,14}. A similar segregational advantage is also possible with sex^{11,16-18}. However, recombination is irrelevant without sex, because unique mutations in different copies of the genome are distributed independently even without it.

The asexual ploidy cycle may cause a substantial decrease of the load only if $NU \sim 1$ or higher, because otherwise the load is always low. Because this cycle must incur some cost in terms of time and energy, it can hardly evolve if $NU \ll 1$. In some unicellular eukaryotes $U \sim 0.003$ (ref. 19), so that under constant selection the decrease of the load can be important only if $N > 100$. If, however, selection acts only once in T generations, TU should be used instead of U , and if $TU \geq 1$ a ploidy cycle every T or more generations may evolve even in diploids. Thus, if intensity of selection fluctuates, the asexual ploidy cycle may be possible even if $U \ll 1$ and haploidy is never favoured directly. Even if $NU > 1$, under a weak selection a rare ploidy cycle is sufficient for most of the possible decrease of the load (Fig. 1), and the cost may preclude it from becoming more frequent.

Among extant asexuals, ploidy cycles *sensu stricto* (cyclical polyploidy²) with different cytological mechanisms are known in *Pyronympha flagellata*², *Entamoeba invadens*²⁰, *Amoeba proteus*²¹, and the radiolarian classes Phaeodaria and Polycystina². *Pelomyxa palustris*¹⁰ has a karyonic cycle. The episodes of low ploidy are always short, which is to be expected if they appear only as a part of the mutation load-decreasing mechanism. More cases may await discovery. Variability of the ploidy of a single nucleus (*Euglena gracilis*², *Holomastigotoides psammotermittides*²) or of the number of nuclei (rhizopods *Parachaos zooclorelae*²², *Gruberella flavescens*²³ and *Euhyperamoeba fallax*²³) has been observed in some other unicellular asexuals, without direct evidence of endomitosis or reduction.

Several more asexual forms are known to be polyploid (*Giardia lamblia*²⁴, *Entamoeba histolytica*²⁰ and *Acanthamoeba castellanii*²⁰), although variations of the ploidy level were not reported. Without a mechanism that preserves similarity among the genomes, long-term diploidy or polyploidy under asexuality leads to their profound divergence under mutation pressure, as in the case of two genomes of bdelloid rotifers (M. Meselson, personal communication). But all 6–10 genomes of *Giardia lamblia* are very similar²⁴, suggesting operation of a homogenizing mechanism, perhaps a ploidy cycle. Alternatively, multiple genomes can replicate asynchronously, like organelle genomes²⁵. This is roughly equivalent to a ploidy cycle every $\sim 2N$ generations and thus also reduces the load²⁶. However, this can cause aneuploidy, unless all the chromosomes of a genome³ replicate simultaneously, for example if each genome occupies its own nucleus.

Asexuality of some of these forms, particularly of Phaeodaria and Polycystina, whose complete life cycles remain obscure¹⁰, is uncertain. However, *Giardia* and *Entamoeba*, which lack mitochondria and diverged from the rest of eukaryotes very early^{27,28}, are perhaps primitively asexual²⁹. Some sexual forms also have similar ploidy cycles (*Opalinata*¹⁰, *Granuloreticulosa*¹⁰, *Ciliophora*^{2,3}, *Ectocarpus siliculosus*³⁰ and probably *Gracilaria*⁴), in addition to their basic alternation of haploid and diploid phases.

The ploidy cycle in an ancestral asexual form may have facilitated the origin of sex by providing the pre-existing mechanism of regular reduction. Reduction must appear almost immediately after syngamy³¹, and without such a mechanism it could often lead to aneuploidy³². If syngamy is followed by karyogamy, only the asexual ploidy cycle *sensu stricto* can provide a suitable mechanism, and only if after endomitosis homologous chromosomes become at least briefly separated, because after syngamy they come from different cells. However, if primitive sex involved no karyogamy and consisted of fusion of polynuclear cells followed by a cell division after which each daughter cell received

nuclei from both parents³³, the karyonic cycle may also have facilitated its origin. This form of gene exchange, which may also reduce the load, has been observed in an 'asexual' *Hyperamoeba fallax*³⁴ and others³³. Further data on the ploidy cycles in unicellular eukaryotes will shed more light on the origin of sex. □

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Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor

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PLATELET-ACTIVATING factor (PAF) is involved in a variety of biological and pathological processes¹ and PAF acetylhydrolase, which inactivates PAF by removing the acetyl group at the *sn*-2 position, is widely distributed in plasma and tissue cytosols^{2,3}. One isoform of PAF acetylhydrolase present in bovine brain cortex is a heterotrimer comprising subunits with relative molecular masses of 45K, 30K and 29K (ref. 4). We have now isolated the complementary DNA for the 45K subunit. Sequence analysis revealed a striking identity (99%) of the subunit with a protein encoded by the causative gene (*LIS-1*) for Miller-Dieker lissencephaly⁵, a human brain malformation manifested by a smooth cerebral surface and abnormal neuronal migration. This indicates that the *LIS-1* gene product is a human homologue of the 45K subunit of intracellular PAF acetylhydrolase. Our results raise the possibility that PAF and PAF acetylhydrolase are important in the formation of the brain cortex during differentiation and development.

PAF acetylhydrolase in bovine brain was chromatographically separated cytosol into three isoforms. We purified one isoform which consists of three polypeptide subunits with relative molecular masses of 45K, 30K and 29K. The 29K subunit acts as a catalytic centre⁴, but the functions of the other subunits are so far unknown. We therefore isolated the cDNA encoding the 45K subunit, with a view to determining its function.

The isolated cDNA contains an open reading frame of 410 amino acids, which includes the sequence of the N terminus and all peptide fragments, so confirming the identity of the clone (Fig. 1); there are also 843 base pairs (bp) of 5' untranslated region upstream and a long 3' untranslated region of 3,695 bp.

The latter region contains eight copies of AATAAA, a polyadenylation signal, and eleven copies of the ATTAA motif, which has been shown to destabilize the messenger RNA when present in the 3' untranslated region of certain cytokines, transcription factors and proto-oncogenes⁶. The predicted primary structure of the 45K subunit matches that of the causative gene (*LIS-1*) for human Miller-Dieker lissencephaly (MDL) (ref. 5). Of the 410 amino-acid residues in bovine brain PAF acetylhydrolase, 407 are identical to those in the human *LIS-1* protein, indicating that the *LIS-1* product could be a human homologue of a subunit of bovine brain PAF acetylhydrolase.

MDL is a brain malformation manifested by a smooth cerebral surface and showing microscopic evidence of incomplete neuronal migration. The *LIS-1* gene was originally isolated using the polymerase chain reaction (PCR) during an attempt to find genes containing repeats found in the β -subunits of G proteins⁷, and was eventually identified as the causative gene for MDL as it is deleted in most patients⁸. The *LIS-1* protein was assumed to be involved in signal transduction because of its homology with the β -subunit of trimeric G proteins: it has seven G β (or WD-40) repeats. More than 20 proteins are now known to contain similar repeating units, but the members of this family do not share any obvious function. They may form essential components of large protein complexes (such as G proteins, PRP4, Tup1 and TFIID⁹), rather as the 45K subunit (or *LIS-1* protein) forms part of the trimeric complex of PAF acetylhydrolase.

When applied to a column of heparin or sulphated cellulose, purified PAF acetylhydrolase was absorbed at neutral pH (data not shown), but under mildly acidic conditions (pH 6.0) most of the original activity passed through the column (Fig. 2a). SDS-PAGE of the fractions revealed that the 29K/30K doublet bands co-migrated precisely with the enzyme activity, whereas the 45K band was recovered in a region of no activity (Fig. 2b). The relative molecular masses estimated for the enzyme before and after heparin chromatography were ~100K and ~50K, respectively (Fig. 2c). We conclude that the 45K polypeptide is dissociated from the native complex on a heparin column under mildly acidic conditions, and that it may not be essential for catalytic activity. The whole enzyme could bind to heparin through interaction of the 45K subunit with polyanions. The 29K/30K complex released from the 45K subunit still had full PAF acetylhydrolase activity, suggesting that the 45K subunit might influence PAF acetylhydrolase activity regulating its interaction with unknown cellular components.

Evidence is accumulating that PAF serves as a transmitter in the central nervous system: it is produced in nervous tissues

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through electrical convulsant stimuli, and upon application of acetylcholine or dopamine¹⁰; the PAF receptor¹¹ is present in the central nervous system¹²; PAF induces phosphoinositide turnover or an increase in the intracellular Ca^{2+} concentration in several cultured neuronal cell systems¹³; and it serves as part

of a retrograde signalling cascade in long-term synaptic potentiation associated with formation of memory¹⁴. Lissencephaly is the direct result of incomplete migration of immature neurons¹⁵ to the cerebral cortex during the third and fourth gestational months. Although the cause of the neuronal migration defect is

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844 ATGGTGTCTGCCAGAGACAACGAGATGAACATAATCGAGCTATAGCAGATTATCTTCGT
M V L S O R O R D F L N R A I A D Y (U) R 20
904 TCAAATGGCTACGAAGAAGCATATTCAGTTTAAAAAGGAAGCTGAATTAGATATGAAT
S N G Y E E A Y S V F K K E A E L D (M) N 40
964 GAAGAATTAGATAAGAAATATGCTGGTCTTTGGAAAAAATGGACATCTGTATTAGA
E E L D K K Y A G L L E K K W T S V I R 60
1024 TTACAAAAGAGGTTATGGAATAGAATCAAGTTAAATGAAGCAAAAGAAATATTCAG
L Q K K V M E L E S K L N E A K E E F T 80
1084 TCGGGTGGACCTCTTGGTCAGAAAAGAGACCCAAAGAAATGGATTCCCGTCCACAGAA
S G G P L G Q K R D P (K) E W I P R P P E 100
1144 AAATATGCATTGAGTGGTCATAGGAGTCCAGTCTCGAGTCAATTTCCATCTGTGTTC
K Y L L S G H R S P V T R V I F H P V F 120
1204 AGTGTATGTCTCTGCTTCAGAGGATGCTACAAATAGGTGTGGGATTATGAGACTGGA
S V M V S A S E D A T I K V W D Y E T G 140
1264 GATTTTGAACGAACCTTTAAGGGGATACAGACTCTGTACAGGATATTTTCATTCGACCAC
D F E R T L K G H T D S V Q D I S F D H 160
1324 AGTGGCAAGCTTCTGGCTTCATGTTCTGCAGATATGACCATTAAGCTATGGGATTTTCAG
S G K L L A S C S A D M T I K L W D F Q 180
1384 GGCTTTGAATGCATCAGAACCATGCATGGCCATGACCAATGTTCTTCAGTACCCATC
G F E C I R T M H G H D H N V S S V A I 200
1444 ATGCCCAATGGAGATCATATAGTGTCTGCCTCAAGGGATAAACTATAAAATGTGGGAA
M P N G D H I V S A S R D K T I K M W E 220
1504 GTGCAACTGGCTACTGTGGAAGACATTCACAGGACAGAGAATGGGTACGATGGTG
V Q T G Y C V K T F T G H R E W V R M V 240
1564 CGGCCAATCAAGAGCGCACTCTGATAGCCAGCTGTTCCAATGACCAAGCTGTGGGTGA
R P N O D G T L I A S C S N D Q T V R V 260
1624 TGGGTCTGTAGCAACAAGGAATGCAAGGCTGAGCTTCGAGAACATGAGCATGTGGTAGAA
W V V A T K E C K A E L R E H E H V E 280
1684 TGCATTTCTGGGGCTCTGAAGGCTCATATTTCTCCATCTCTGAAGCAACAGGATCTGAG
C I S W A P E S S Y S S I S E A T G S E 300
1744 ACTAAAAAAGTGGCAACCTGGGCCATCTTACTGTCCGGATCCAGGACAGACTATC
T K K S G K P G P F L L S G S R D K T I 320
1804 AAGATGTGGGATGTGAGTACTGGCATGTGCCTATGACCCCTGGTGGGTCATGATACTGG
K M W D V S T G M C L M T L V G H D N W 340
1864 GTACGTGGAGTTCTGTTCATCTCTGGGGGAAGTTATTTTGTAGTTGCGCTGATGACAAG
V R G V L F H S G G K F I L S C A D D K 360
1924 ACCCTGCGGTGTGGGATTACAAGACAAGCGATGCATGAAGACCCCTCAATGCGCATGAA
T L R V W D Y K N K R C M K T L N A H E 380
1984 CACTTTGTACCTCTTGGATTTCATTAAGACGGCCCATATGTGGTTACTGGCAGTGTA
H F V T S L D F H K T A P Y V V T G S V 400
2044 GATCAACAGTAAGGTGTGGGAGTGTCTGTGA
D Q T V K V W E C R * 410

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FIG. 1 Nucleotide and predicted amino-acid sequences of the coding region of the cDNA encoding the 45K subunit of bovine brain PAF acetylhydrolase. Nucleotides are numbered on the left and amino acids on the right. The termination codon is denoted by an asterisk. Different residues from the *LIS-1* product are circled: in *LIS-1*, Leu 19 and Met 39 are substituted by Pro and Val, respectively, and Lys 92 is deleted. Polyadenylation signals are at nucleotide positions 2,160, 2,350, 2,534, 3,305, 3,559, 4,269, 4,787 and 5,313, and the ATTTA sequence is at nucleotide positions 2,265, 2,764, 3,657, 3,673, 3,804, 4,293, 4,716, 4,779, 5,234, 5,253 and 5,499. This nucleotide sequence has been submitted to the GenBank/EMBL Data Bank under accession number D30615.

METHODS. Bovine brain PAF acetylhydrolase was purified according to ref. 4. The 45K subunit was prepared as described in Fig. 2 legend, purified by reverse-phase HPLC, reduced with dithiothreitol, S-alkylated with 4-vinylpyridine, and then proteolysed with lysyl-endopeptidase. Peptide fragments were isolated by reverse-phase HPLC. Three partial amino-acid sequences and the N-terminal amino-acid sequence (underlined) were obtained. Two degenerate oligonucleotides were synthesized (where N is A, C, G or T; R is A or G; Y is C or T; and K is A or C): GGNTAYGARGARGCNTA, based on the amino-acid sequence GYEEAY, and TGRITNGGNCKNACCAT, based on the amino-acid sequence MVRPNQ. PCR was carried out on bovine brain cDNA using these primers. The amplified product (665 bp) was sequenced and, on the basis of the sequence, specific primers (AAGAGACCCAAAAGATG and GCATCTCCACATTTT) were synthesized for further screening of the library using PCR^{17,18}. Briefly, the cDNA plasmid library was distributed into 96-well plates (50 clones per well), and the supernatants were pooled in every column and row. PCR was used to detect the wells containing positive clones. Positive pools were plated and every colony was again checked by PCR with specific primers. The nucleotide sequence revealed one open reading frame which contained all the partial amino-acid sequences.

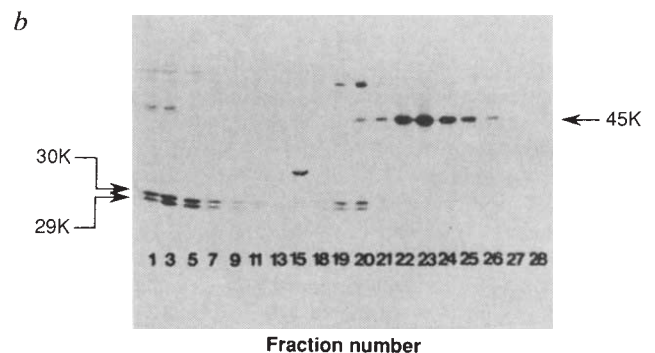
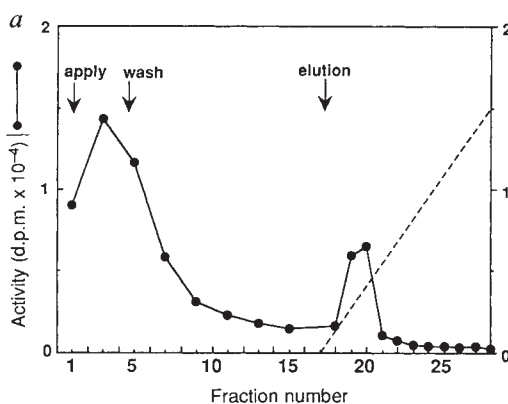
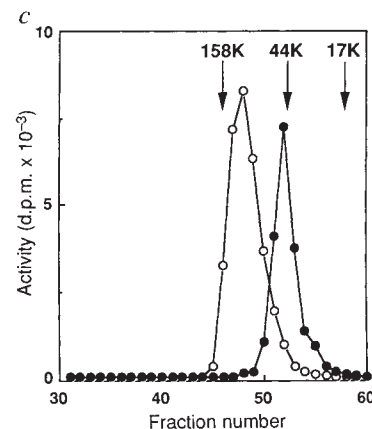


FIG. 2 Separation of the 45K subunit from the 30K/29K complex on a heparin-Sepharose column. *a*, Elution profile of PAF acetylhydrolase activity on heparin-Sepharose. PAF acetylhydrolase was partially purified from bovine brain as described⁴ (using active fractions after the hydroxyapatite step). The sample was dialysed against buffer A (10 mM KH_2PO_4 -KOH, pH 6.0, 5 mM 2-mercaptoethanol and 10% glycerol) and then applied to the heparin-Sepharose column (1.0 $\times$ 7.0 cm) equilibrated with buffer A. After washing with buffer A, the column was eluted with 40 ml of a linear gradient of NaCl (0–1.5 M) in buffer A. Fractions (4 ml) were collected and examined for PAF acetylhydrolase activity as described⁴. *b*, SDS-PAGE (12%) and silver staining heparin-Sepharose fractions. *c*, Elution profile of PAF acetylhydrolase on Superose 12 HR 10/30 FPLC gel filtration. The active fraction (100 μ l) before (circles) or after (filled circles) heparin-Sepharose chromatography was applied to a column equilibrated with buffer A; fractions (0.25 ml) were collected and tested for activity. Molecular size markers were γ -globulin (158K), ovalbumin (44K) and myoglobin (17K).



not yet known, the abnormality may exist in a signal which initiates or continues migration, or in the ability of the neurons to climb the radial glia efficiently. It has been reported that exposure of cultured neuronal cells to low concentrations of PAF induces differentiation, whereas a high concentration of PAF is neurotoxic¹⁶. These observations show that adjustment of the PAF concentration within a certain range may be crucial for the progress of differentiation.

A future challenge will be to determine how PAF or PAF acetylhydrolase is involved in the process of neuronal cell differentiation or brain development. □

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Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing

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TUMOUR necrosis factor (tumour necrosis factor- α /cachectin) plays a critical role in certain physiological defensive responses but causes severe damage to the host organism when produced in excess¹. There are two forms of tumour necrosis factor, a type II membrane protein of relative molecular mass 26,000 (26K) and a soluble, 17K form generated from the cell-bound protein by proteolytic cleavage^{2,3}. The two forms of tumour necrosis factor and lymphotoxin- α (tumour necrosis factor- β /lymphotoxin), a related protein, have similar but apparently not identical biological activities^{4–6}. A therapeutic agent which inhibited the release of tumour necrosis factor, but did not reduce the cell-associated activity or the level of lymphotoxin- α , might preserve the benefits of these cytokines while preventing tumour necrosis factor-induced damage. Here we describe a potent inhibitor of tumour necrosis factor processing and report that it protects mice from a lethal dose of endotoxin.

The tumour necrosis factor (TNF) released by the monocytic cell line THP-1 was found to begin with Val 77 of the precursor, consistent with previous studies using other TNF-producing cells^{7,8}. A pulse-chase study with the THP-1 cells showed no intermediate forms of the cytokine and suggested that processing occurs at the cell surface (data not shown).

On the basis of these observations, we looked for a TNF-processing activity in the membranes of THP-1 cells. Recombinant FLAG-precursor TNF (see Fig. 1 legend) was incubated with putative protease-containing samples, and cleavage was detected by western blot analysis. The recombinant precursor

was susceptible to physiologically irrelevant cleavages, which were eliminated by diethylaminoethyl (DEAE) fractionation and incubation in the presence of protease inhibitors that do not block the release of TNF from cells. DEAE fractions that eluted with 50–150 mM NaCl generated a 17K form of TNF (Fig. 1) which begins with Val 77, whereas a major enzyme activity that cleaved within the mature domain of the precursor did not bind to the DEAE column. The specificity of the partially purified protease was further tested by incubating it with a 20-residue peptide representing Leu 67 to Asp 86 of full-length TNF; only two products were generated and analysis by mass spectrometry showed that these products resulted from a cleavage between the residues corresponding to Ala 76 and Val 77.

To tailor an inhibitor for this protease, we first determined the mechanistic class to which the enzyme belongs by testing its

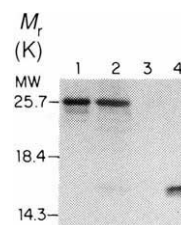


FIG. 1 Generation of 17K TNF by a preparation of THP-1 membrane proteins. THP-1 membrane proteins were solubilized, fractionated and treated as described below. Proteins that eluted with 100 mM NaCl were used in this experiment. The mixtures indicated below (lanes 1–3) were incubated at 37 °C for 4 h and were then analysed by western blot. 1, FLAG-precursor TNF plus buffer; 2, FLAG-precursor TNF plus protease preparation; 3, protease preparation plus buffer; 4, recombinant TNF (R & D Systems).

METHODS. THP-1 cells were stimulated as previously described¹⁹ and plasma membranes were isolated by method 3 of ref. 20, except that dithiothreitol was not included. Protein was solubilized by resuspending the membrane preparation in a solution of 1% octylglucoside, 10 mM Tris-HCl (pH 8), 1 mM MgCl₂, 30 mM NaCl and briefly homogenizing. After centrifugation, the supernatant fraction was diluted 2.5-fold with 1% octylglucoside, 10 mM Tris-HCl (pH 7.5), and applied to a column of DEAE-Sephacel. Proteins were eluted with an increasing gradient of NaCl (0–0.3 M). Eluted material was incubated with FLAG-precursor TNF in the presence of 1 mM N-methoxysuccinyl-Ala-Ala-Val-chloromethylketone, 10 µg ml⁻¹ leupeptin, and 1 mg ml⁻¹ α -1-protease inhibitor. 'FLAG' is a sequence of eight amino acids which has been fused to the amino terminus of a variety of proteins to help expression and purification²¹. DNA encoding human precursor TNF was spliced to DNA encoding the FLAG sequence, and this construct was inserted into plasmid pL3 (ref. 22). The protein was then expressed in a protease-deficient strain of *Escherichia coli*²³. It was extracted from the insoluble fraction of a bacterial lysate with 8 M urea and then dialysed against 10 mM Tris-HCl (pH 8). Western analysis was as previously described²⁴ except that the first antibody used was a rabbit polyclonal antiserum raised against recombinant 17K TNF.

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sensitivity to a series of class-specific inhibitors (Table 1). The only effective inhibitor in this series was EDTA, which chelates cations associated with metalloenzymes, and the inclusion of Zn^{2+} with this compound restored activity. The enzyme thus appears to be a metalloprotease. We then screened a panel of more specific metalloprotease inhibitors and found a hydroxamic acid (compound 1) which effectively blocked the processing activity (Table 1). The 50% inhibitory concentration (IC_{50}) of this compound in the TNF peptide cleavage assay was about 100 nM.

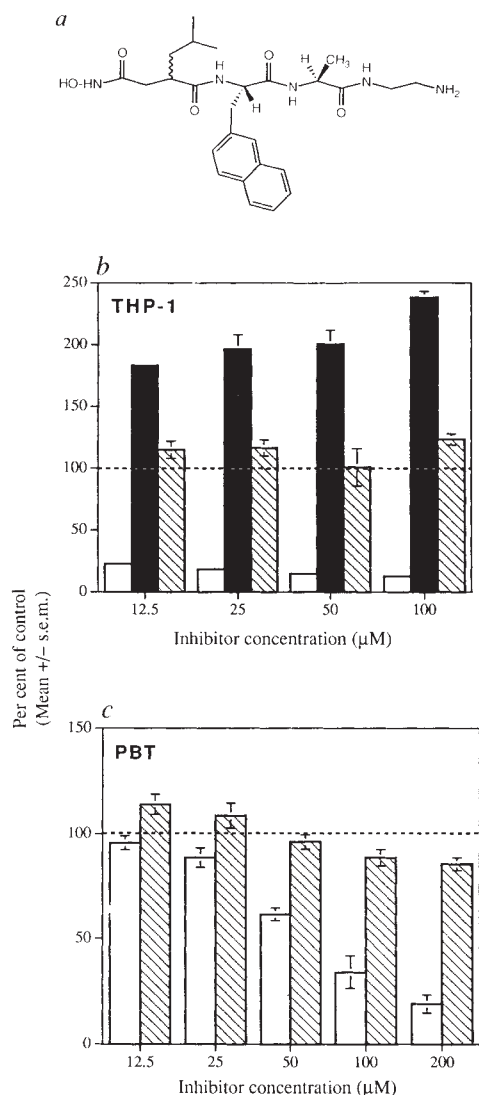


FIG. 2 An inhibitor of the processing enzyme blocks the release of TNF but not of other cytokines. **a**, Structure of compound 2: N -[D,L-[2-(hydroxyaminocarbonyl)methyl]-4-methylpentanoyl]-L-3-(2'-naphthyl)-alanyl-L-alanine, 2-aminoethyl amide. The two diastereomers were separated by reverse-phase HPLC, using a C_{18} column and a 2% min^{-1} gradient of acetonitrile, 0.1% trifluoroacetic acid. The active diastereomer is the later-eluting form. **b**, THP-1 cells were stimulated with $1 \mu\text{g ml}^{-1}$ lipopolysaccharide for 2 h in the presence of the indicated concentrations of compound 2. Cell-associated TNF was solubilized with 1% Triton X-100 before assay. Cytokine levels were measured in duplicate by ELISA. White bar, TNF in medium; solid bar, cell-associated TNF; hatched bar, IL-8 in medium. The increase in cell-associated TNF accounted for less than 10% of the reduction in the soluble form. **c**, Human peripheral blood T cells (PBT) were stimulated for 24 h with plate-bound OKT3 monoclonal antibody and phorbol myristate acetate (10 ng ml^{-1}) in the presence of the indicated concentrations of compound 2. Cytokine levels were assayed in duplicate by ELISA. White bar, TNF in medium; hatched bar, lymphotoxin- α in medium.

TABLE 1 Metalloprotease inhibitors block TNF processing activity

Inhibitor (concentration)	Class inhibited	Per cent of control
PMSF (1 mM)	Serine	92
α -1-Pi (1 mg ml^{-1})	Serine	97
Iodoacetate (1 mM)	Cysteine	96
Pepstatin (10 μM)	Aspartyl	106
EDTA (5 mM)	Metallo	0
EDTA (1 mM)		8
EDTA (1 mM) + 1 mM ZnCl_2		100
Compound 1 (1 μM)		30

The processing activity eluted from the DEAE column was further purified by wheat-germ agglutinin-agarose chromatography. The activity was assayed by the extent of cleavage of an eight-residue peptide spanning the processing site. The control in each case was the buffer in which the inhibitor was dissolved. The values shown are the average of duplicate assays for each condition. PMSF, phenylmethanesulphonyl fluoride; α -1-Pi, α -1-protease inhibitor; EDTA, ethylenediaminetetraacetic acid; compound 1, N -[D,L-[2-(hydroxyaminocarbonyl)methyl]-4-methylpentanoyl]-L-3-(2'-naphthyl)-alanyl-L-alanine amide¹⁵. The compound 1 used in the experiments reported here was made by a novel synthetic procedure (manuscript in preparation). This compound inhibits gelatinase and collagenase. It is not clear why compound 1 inhibits the TNF-processing enzyme, because there is no apparent similarity between the cleavage sites in collagen¹⁶ and 26K TNF. Several lines of evidence suggest the protease is not collagenase or one of the other matrix-degrading metalloproteases¹⁷: the TNF-processing enzyme does not bind to heparin, does not require Ca^{2+} and is not inhibited by tissue inhibitor of metalloproteases. The enzyme is also resistant to phosphoramidon and captopril, inhibitors of other well-characterized metalloproteases¹⁸.

To test whether a metalloprotease is in fact used by cells to release TNF, we used another hydroxamate (compound 2; Fig. 2a) similar in efficacy *in vitro* to compound 1 but more stable in serum. This inhibitor caused a dose-dependent reduction in the level of TNF in the medium of both THP-1 cells and peripheral blood T cells (Fig. 2b, c). This effect was not due to a general reduction in secretion, because the inhibitor caused no decrease in the amount of interleukin-8 (IL-8) secreted by the THP-1 cells and only a slight reduction in the amount of lymphotoxin- α secreted by the T cells. Moreover, compound 2 caused a dose-dependent increase in the level of THP-1 cell-associated TNF (Fig. 2b), consistent with an inhibition of release but not of synthesis, and the level of TNF messenger RNA in both cell types (determined by northern blots) was unchanged by the inhibitor (data not shown). To determine the effect of compound 2 on cell-associated TNF in T cells, we used a human CD4^+ T-cell clone stimulated with phorbol myristate acetate and ionomycin, and as a measure of specificity we also determined the inhibitor's effect on two other members of the TNF ligand family. Fusion proteins consisting of the Fc portion of a human IgG1 molecule coupled to the extracellular portion of the TNF receptor (TNFR; p80)⁹, 4-1BB¹⁰ or CD40 (ref. 11), were used to detect the cell-surface ligands by fluorescence-activated cell sorting (FACS) analysis¹². The mean fluorescent intensities observed with the TNFR-Fc were 3,040 and 83 (arbitrary units) in the presence (200 μM) and absence of the inhibitor, respectively. The corresponding values for 4-1BB-Fc were 423 and 344, and for CD40-Fc the value was 107 both with and without the inhibitor. This experiment provides the most conclusive evidence that compound 2 blocks processing because it measured cell-surface rather than total cell-associated TNF, which could be increased by other mechanisms such as blocking transport to the plasma membrane.

The efficacy and specificity of compound 2 in tests with cells in tissue culture prompted us to test its effect *in vivo*. We first determined whether the inhibitor would block the rise in serum TNF normally observed when mice are injected with lipopolysaccharide (LPS). In an experiment in which the level of TNF

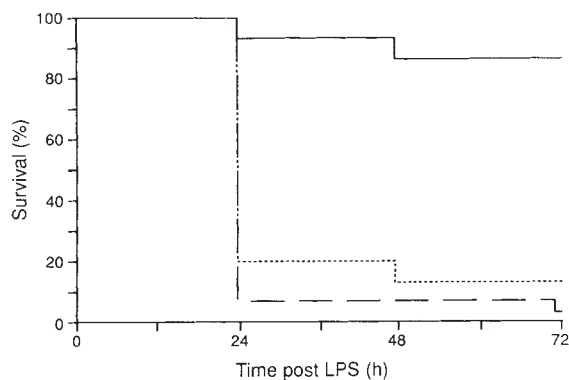


FIG. 3 Treatment with a TNF-processing inhibitor prevents mortality induced by LPS plus D-galactosamine. BALB/c mice (18–20 g) received 20 mg of D-galactosamine and 20 ng of LPS intravenously. Immediately thereafter, compound 2 (1 mg) or an equivalent volume of saline (0.5 ml) was administered subcutaneously. The results shown are a compilation of four experiments using 9–10 mice per treatment group for each experiment. Solid line, compound 2 treatment; dashed line, saline treatment; dotted line, no treatment. Additional experiments demonstrated that treatment with 0.5 mg of compound 2 was suboptimal (4/30 mice (13%) survived), whereas 100% survival was achieved by treatment with 1.5 mg of compound 2 (10/10 mice).

in the sera of control mice (injected intravenously with 450 μ g LPS and then subcutaneously with a saline solution) was 1,732 pg ml^{-1} , 500 μ g of compound 2 injected at the time of LPS administration reduced serum TNF to 281 pg ml^{-1} (sera were pooled from two mice for each treatment 2 h after injection, and TNF levels were determined by enzyme-linked immunosorbent assay (ELISA); similar results were obtained in two additional experiments). A diastereomer of compound 2 (Fig. 2), which was at least 10-fold less active than compound 2 against the partially purified enzyme *in vitro*, caused no reduction in serum TNF levels. We then tested whether the inhibitor would save mice from a dose of LPS plus D-galactosamine that induces a lethal TNF-mediated response^{13,14}. Administration of 1 mg compound 2 at the time of challenge increased the rate of survival (Fig. 3). An inhibitor of TNF processing can thus act as an effective therapeutic agent *in vivo*. More specific inhibitors will be required to determine whether this beneficial effect is due solely to the inhibition of TNF release. □

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p53-Dependent apoptosis in the absence of transcriptional activation of p53-target genes

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THE tumour suppressor p53 is required to induce programmed cell death (apoptosis) by DNA-damaging agents^{1,2}. As p53 is a transcriptional activator³ that mediates gene induction after DNA damage⁴, it has been proposed to be a genetic switch that activates apoptosis-mediator genes⁵. Here we evaluate the role of p53 in DNA-damage-induced apoptosis by establishing derivatives of GHFT1 cells, that are somatotropic progenitors immortalized by expression of SV40 T-antigen⁶, which express a temperature-sensitive p53 mutant⁷. In these cells induction of apoptosis by DNA damage depends strictly on p53 function. A shift to the permissive temperature triggers apoptosis following DNA damage, but this is independent of new RNA or protein synthesis. The extent of apoptotic DNA cleavage is directly proportional to the period during which p53 is functional. These results do not support the proposal that p53 is an activator of apoptosis-mediator genes but rather indicate that p53 either represses genes necessary for cell survival⁸ or is a component of the enzymatic machinery for apoptotic cleavage or repair of DNA⁵.

The p53-Vall35 temperature-sensitive (ts) allele⁷ was introduced into GHFT1 cells derived from immortalized somatotropic progenitors using a GHFT1-T antigen (Tag) transgene⁶. As Tag immortalizes cells by direct binding to the p53 and retinoblastoma (Rb) tumour suppressors^{9,10}, we increased the level of p53 in GHFT1 cells using the temperature-sensitive p53 allele in order to control their growth by modulating p53 activity as a result of temperature shift⁷. Three stably transfected clones, GT559, GT433 and GTB23, which expressed more p53 than the parental cells (Fig. 1a), were selected. In agreement with previous results^{11,12}, at the non-permissive temperature (38 °C) the p53-Vall35 protein was mainly cytoplasmic, but was predominantly nuclear at the permissive temperature (32.5 °C) (Fig. 1b). [³H]thymidine incorporation dramatically decreased after shifting the three clones to 32.5 °C (Fig. 1c). This effect was not observed with the parental cells and was directly related to the level of p53-Vall35 expression. Inhibition of DNA synthesis was reversed on returning to 38 °C (data not shown). These results indicate that p53-Vall35 assumes a native conformation at the permissive temperature and counteracts the proliferative action of Tag. To confirm this, we determined the amount of p53 complexed to Tag using monoclonal antibodies against each protein. One of the anti-p53 antibodies (Pab246) reacts only with the native protein¹³, the other (Pab122) reacts with both the native and mutant conformers¹⁴. In GHFT1 cells, some of the Tag was not bound to p53 (compare lanes 1 and 3 in Fig. 1d); in GTB23 cells, which express the lowest amount of p53-Vall35, not all p53 was complexed with Tag (compare lanes 4 and 6 in Fig. 1d). The ratio between free p53 to Tag-bound p53 was higher in GT559 and GT433, which express more p53-Vall35 (data not shown). Conversion of p53-Vall35 to its native conformation correlates with its decreased stability¹¹. Unlike Tag-bound p53, whose half-life of >6 h is not affected by the temperature shift, free p53 has a shorter half-life at the permissive temperature (3 h at 32.5 °C as opposed to 6 h at 38 °C) (data not shown).

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Having established cells whose proliferation depends on p53, we investigated the role of p53 in apoptotic cell death. We used ultraviolet radiation to damage DNA and monitored internucleosomal DNA cleavage as an indicator of apoptosis¹⁵. After exposure to ultraviolet light, cells expressing p53-Val135 displayed internucleosomal DNA cleavage only when kept at 32.5 °C (Fig. 2a). No degradation was seen in the parental cells under any conditions. Unlike subclones of M1 myeloid cells expressing p53-Val135, whose shift to the permissive temperature is sufficient to induce apoptosis¹⁶, apoptotic DNA cleavage in the three somatotropic cell clones was dependent only on DNA damage. The appearance of other features of apoptosis (for example, chromatin condensation and nuclear collapse¹⁷) was also dependent on both ultraviolet irradiation and p53 function (Fig. 2b). Other DNA-damaging agents such as γ -radiation,

mitomycin C and 4-nitroquinoline-1-oxide also induced apoptosis in these cells, but only at the permissive temperature (Fig. 2c). Treatment with reagents such as tumour-necrosis factor- α or ionomycin, or serum deprivation, which do not cause DNA damage but can trigger apoptosis in other cell types⁸, failed to induce it in this system (data not shown).

We further investigated p53 function through temperature-shift experiments. The DNA-degradation ladder became evident 3 h after irradiation with ultraviolet, reaching a plateau at 8–10 h (data not shown). When cells were collected 8 h after temperature shifts made in both directions during the first 6 h after ultraviolet radiation, the extent of internucleosomal DNA cleavage¹⁸ was proportional to the time during which p53 was active (Fig. 3). Cells irradiated at 38 °C underwent apoptosis when shifted to 32.5 °C during the first 4 h after irradiation, indicating that DNA damage can be sensed long after the initial insult. Hence there is a continuous requirement for native p53 for execution of the apoptotic programme.

Genes activated by p53 include *mdm-2* (ref. 19) and *CIP-1/WAF1* (refs 20, 21). We tested whether p53-mediated apoptosis was dependent on transcription of p53 target genes and the synthesis of new proteins and found that when actinomycin D, a transcriptional inhibitor, or cycloheximide, a protein synthesis

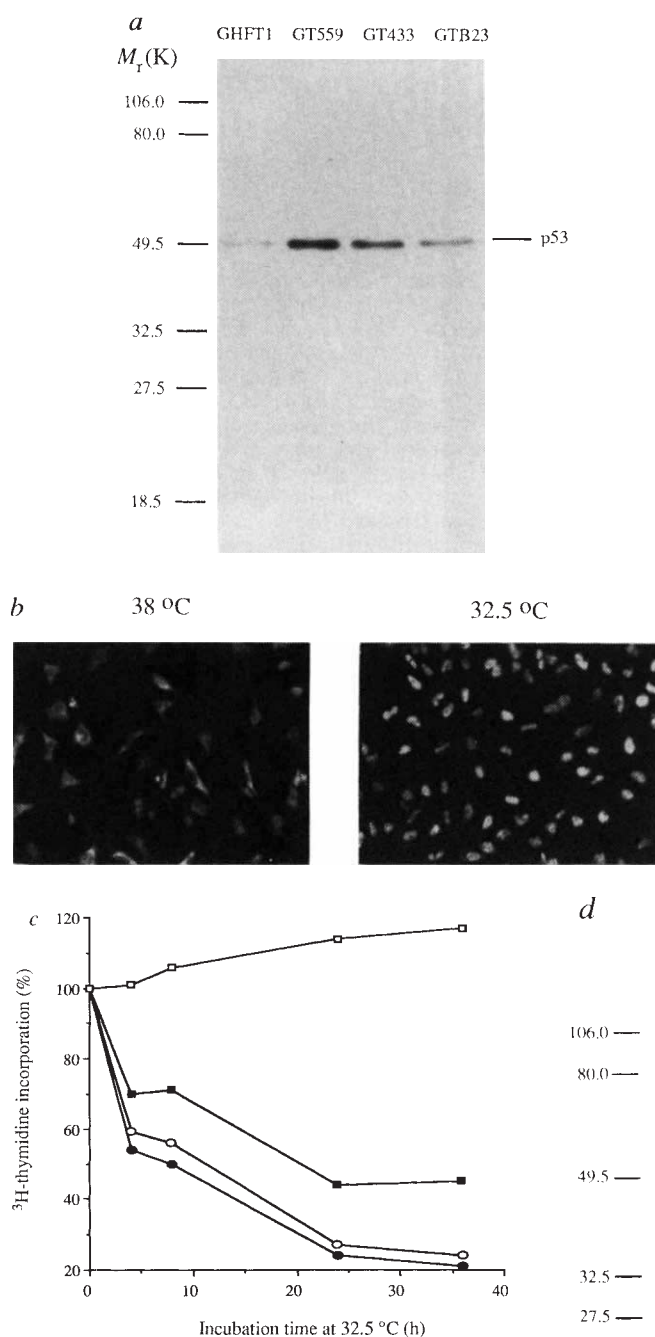


FIG. 1 Characterization of p53-Val135-expressing GHFT1 cells. **a**, Whole cell extracts (20 μ g) of parental GHFT1 cells and p53-Val135-transfected clones GT559, GT433 and GTB23 were analysed by immunoblotting for p53 expression using monoclonal antibody PAb240. **b**, GT559 cells were grown on coverslips for 24 h at 38 °C or 32.5 °C, fixed, and analysed by indirect immunofluorescence using anti-p53 monoclonal antibody PAb122. **c**, Subconfluent cultures of parental GHFT1 cells ($\square$) and clones GT559 ($\bullet$), GT433 ($\circ$) and GTB23 ($\blacksquare$) were grown at either 38 °C or 32.5 °C for different times and then pulsed with [3H]thymidine (2 μ Ci ml⁻¹) for the last 4 h. The percentage of label incorporated into material precipitated by trichloroacetic acid (TCA) (derived from equal numbers of cells) at 32.5 °C versus 38 °C was determined. **d**, Subconfluent GHFT1 and GTB23 cultures maintained at 32.5 °C for 24 h were metabolically labelled with 100 μ Ci ml⁻¹ of Tran³⁵S-label (ICN Radiochemicals) for 2 h. Cells were lysed as described¹³ and equivalent amounts of lysates were immunoprecipitated with either anti-p53 monoclonal antibodies PAb246 (lanes 1, 4) or PAb122 (lanes 2, 5) or anti-Tag monoclonal antibody KT3 (lanes 3, 6) as described¹³. Immunoprecipitates were separated by SDS-PAGE and autoradiographed.

METHODS. Subconfluent GHFT1 cultures were transfected using the calcium phosphate method with 8 μ g pLTRcGval135 (ref. 7) and 2 μ g pRSVneo. G418-resistant clones were selected using 800 μ g ml⁻¹ G418. Individual clones were isolated and 16 were screened by Southern blotting for the ts p53 allele. Of these, 8 were positive and were analysed by northern blotting for p53 mRNA expression to give the three positive clones GT559, GT433 and GTB23.

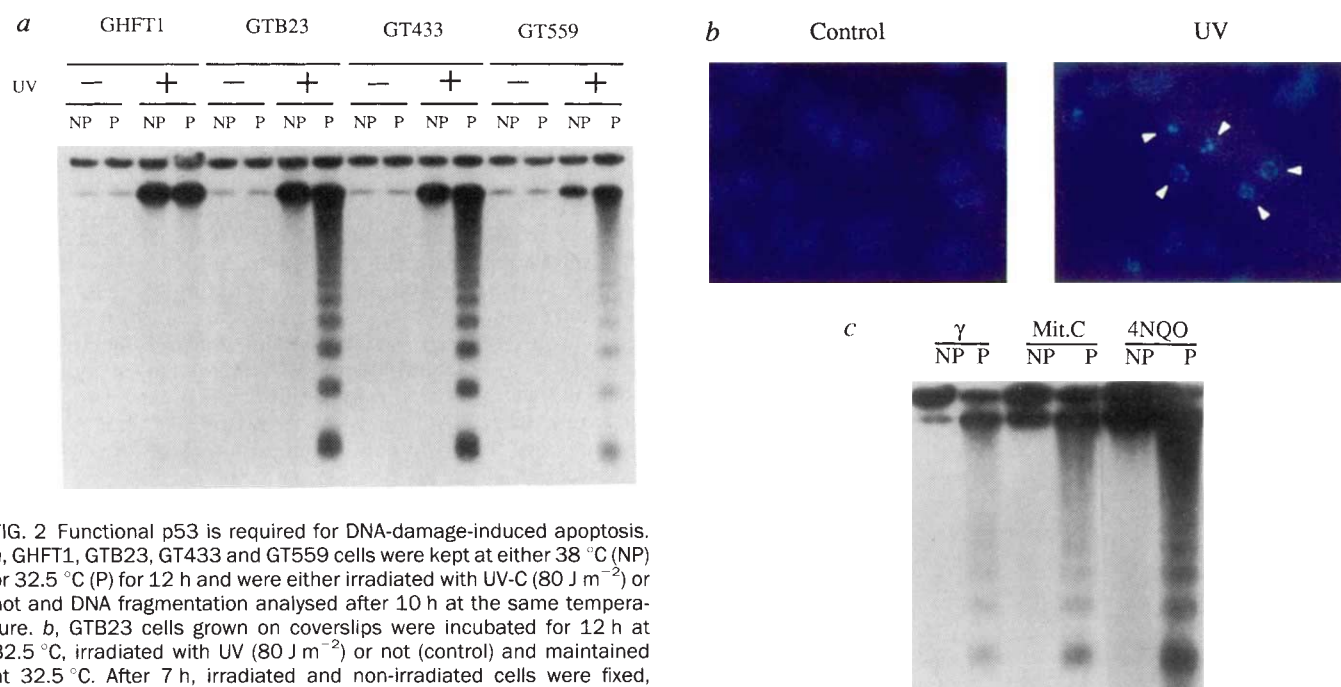


FIG. 2 Functional p53 is required for DNA-damage-induced apoptosis. **a**, GHFT1, GTB23, GT433 and GT559 cells were kept at either 38 °C (NP) or 32.5 °C (P) for 12 h and were either irradiated with UV-C (80 J m⁻²) or not and DNA fragmentation analysed after 10 h at the same temperature. **b**, GTB23 cells grown on coverslips were incubated for 12 h at 32.5 °C, irradiated with UV (80 J m⁻²) or not (control) and maintained at 32.5 °C. After 7 h, irradiated and non-irradiated cells were fixed, stained with 2,4-diamidino-2-phenylindole (DAPI) and surveyed by fluorescence microscopy for cells showing chromatin condensation and nuclear fragmentation (arrowheads). **c**, GT559 cells maintained at either 38 °C (NP) or shifted for 12 h to 32.5 °C (P) were exposed to either 10 Gy of γ -radiation, 10 μ g ml⁻¹ mitomycin C (Mit. C) or 1.5 μ g ml⁻¹ 4-nitroquinoline-1-oxide (4NQO). After culturing for 10 h at 32.5 °C or 38 °C, DNA fragmentation was analysed by end-labelling, agarose gel electrophoresis and autoradiography.

METHODS. 5 $\times$ 10⁵ cells in 60-mm plates were used per point. Before UV irradiation, the medium was removed from both treated and control plates and cell layers washed once with 2 ml prewarmed PBS. After UV irradiation (80 J m⁻²; ref. 19), 1.5 ml prewarmed medium was added and plates were returned to their incubators. All manipulations were

made on prewarmed heating blocks. Cells were scraped into 1.5 ml culture medium, microfuged for 5 min at 13,200 r.p.m., and the pellet was suspended in 300 μ l buffer containing 10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 25 mM EDTA, 0.5% SDS and 0.5 mg ml⁻¹ proteinase K. After overnight incubation at 37 °C, DNA was ethanol-precipitated, suspended in 200 μ l TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) containing 50 μ g ml⁻¹ RNase A, and incubated at 37 °C for 2 h. DNA was extracted and end-labelled using terminal transferase and [α -³²P]-ddATP as described¹⁸. Aliquots of DNA (50 ng) were electrophoresed on 2% agarose gels, which were dried under vacuum and autoradiographed. A ¹³⁷Cs source (Shepherd, Mark I) calibrated by Fricke dosimetry was used to provide 612 rad min⁻¹ γ -radiation.

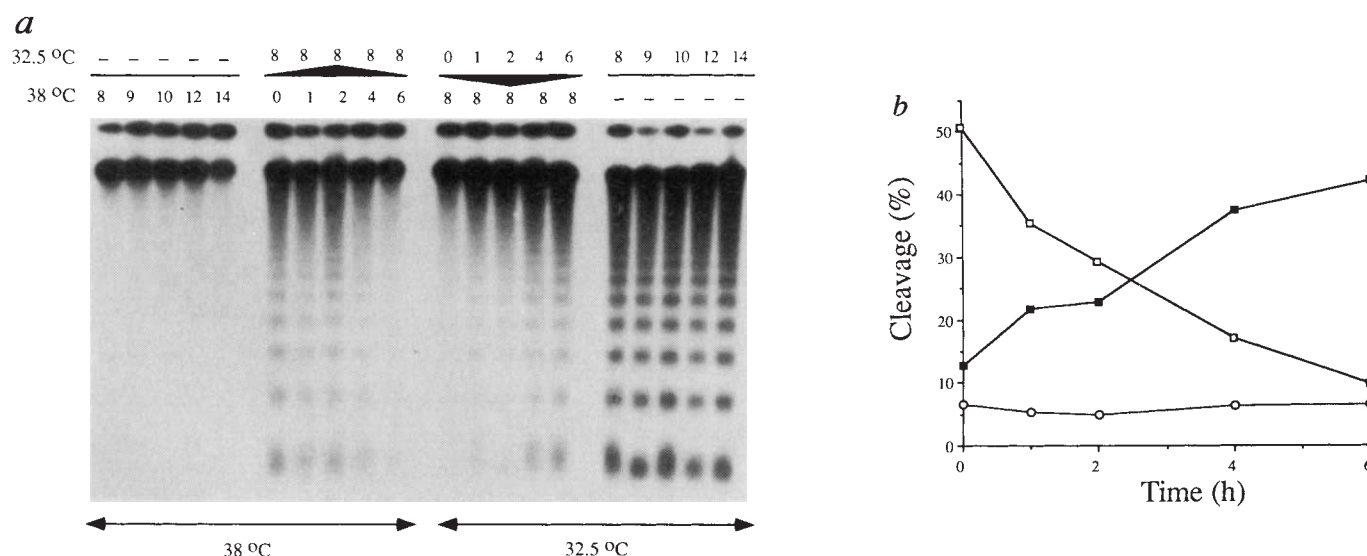


FIG. 3 Continued p53 function is required for execution of the apoptotic programme. **a**, Cultures of GT559 cells (5 $\times$ 10⁵ cells per 60-mm plate) were incubated at 38 °C or 32.5 °C for 12 h, UV irradiated (40 J m⁻²) at the same temperatures, then one plate per point was shifted to the other temperature at 0, 1, 2, 4 or 6 h and kept at that temperature for another 8 h, as indicated, before extraction of genomic DNA. A second set of plates was kept at the irradiation temperature for 8, 9, 10, 12 or 14 h before extraction of genomic DNA. The DNA was end-labelled and analysed as for Fig. 2. **b**, The extent of internucleosomal DNA

cleavage in two independent temperature-shift experiments quantified by densitometry of autoradiograms. Internucleosomal cleavage in cultures was expressed relative to the value in cultures kept for 8 h at 32.5 °C, taken as 100%. These values (averages of two experiments) are plotted as a function of the time at which cultures were shifted to the other temperature. □, Cultures irradiated at 38 °C and shifted at the indicated times to 32.5 °C; ■, cultures irradiated at 32.5 °C and shifted at the indicated times to 38 °C; ○, cultures irradiated at 38 °C and maintained for 8 h plus the indicated times at that temperature.

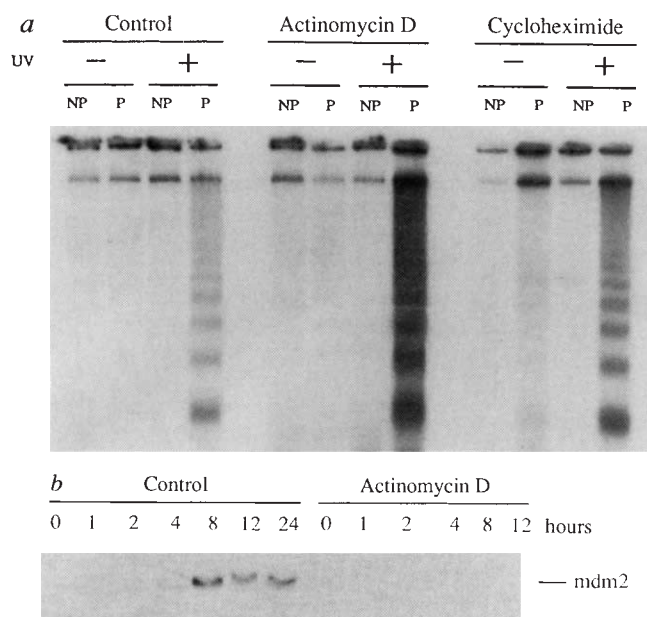


FIG. 4 p53-mediated apoptosis is independent of synthesis of new RNA or protein. **a**, Cultures of GT559 cells (5×10^5 cells per 60-mm plate) were grown at 38°C and treated with either actinomycin D ($2.5 \mu\text{g ml}^{-1}$) or cycloheximide ($10 \mu\text{g ml}^{-1}$), or left untreated. After 1 h, cultures were either not irradiated (–) or UV-irradiated (+) (80 J m^{-2}), then split into two groups, one left at 38°C (NP) and the other one shifted to 32.5°C (P). Drugs were kept in the medium for 9 h before cells were collected, then genomic DNA was extracted and the extent of internucleosomal cleavage determined as for Fig. 2. **b**, Cultures of GT559 cells grown at 38°C were incubated in the absence (control) or presence of actinomycin D ($2.5 \mu\text{g ml}^{-1}$) for 1 h, shifted to 32.5°C and maintained with or without actinomycin D. At the indicated times, total cellular RNA was extracted and 10- μg samples analysed by agarose gel electrophoresis and northern hybridization with an *mdm2* probe.

inhibitor, were added 1 h before irradiation and temperature downshift, the DNA ladder still appeared (Fig. 4a). Cycloheximide induced some apoptotic DNA cleavage on its own, but only at the permissive temperature, even though protein synthesis was $\geq 95\%$ blocked within 30 min, as shown by ^{35}S -methionine incorporation into total cellular protein; also, actinomycin D completely inhibited *mdm-2* induction (Fig. 4b). These results indicate that induction and maintenance of the apoptotic programme in these cells depend on p53 activity and not on synthesis of new RNA or protein, and with our temperature-shifted results, they suggest that p53 is not an activator of apoptosis-mediator genes. We note that the cytostatic effect of p53, probably mediated by CIP-1/WAF1, is much slower than its apoptotic effect (compare Figs 1c and 3).

Overexpression of p53 represses TATA-mediated transcription²². Although our results are consistent with p53 being a repressor of survival genes⁸, as shown for *c-jun* and *c-myc*²³, this repression is transient (data not shown). We did not observe any effect of p53 on *c-jun* and *c-fos* induction as a result of DNA damage²⁴, activation of the ultraviolet-responsive protein kinase JNK²⁵, or of *bcl-2* expression²⁶. Although general inhibitors of gene expression did induce a low level of apoptosis in our system, their effect was dependent on p53. We therefore favour the hypothesis that p53 controls apoptosis as a result of its participation in either DNA repair⁵ or apoptotic DNA cleavage²⁷. In support of this idea, p53 interacts directly with replication protein A (refs 28–30), which is involved in both DNA replication³¹ and repair³². Our conclusions are based on Tag-immortalized cells, but should be applicable physiologically considering that Tag expression in thymocytes blocks only irradiation-induced apoptosis and not clonal deletion, with p53 binding being essential for the former effect³³. □

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Nuclear protein CBP is a coactivator for the transcription factor CREB

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THE transcription factor CREB binds to a DNA element known as the cAMP-regulated enhancer (CRE)^{1–5}. CREB is activated through phosphorylation by protein kinase A (PKA)⁶, but precisely how phosphorylation stimulates CREB function is unknown. One model is that phosphorylation may allow the recruitment of coactivators which then interact with basal transcription factors. We have previously identified a nuclear protein of *M*, 265K, CBP, that binds specifically to the PKA-phosphorylated form of CREB⁷. We have used fluorescence anisotropy measurements to define the equilibrium binding parameters of the phosphoCREB:CBP interaction and report here that CBP can activate transcription through a region in its carboxy terminus. The activation domain of CBP

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interacts with the basal transcription factor TFIIB through a domain that is conserved in the yeast coactivator ADA-1 (ref. 8). Consistent with its role as a coactivator, CBP augments the activity of phosphorylated CREB to activate transcription of cAMP-responsive genes.

The interaction domain of phosphoCREB and CBP has been mapped previously (Fig. 1a). We used measurements of fluo-

rescence anisotropy to quantify how phosphorylation of CREB altered the affinity of this interaction. The fluorescence anisotropy of a fluorochrome-labelled oligonucleotide is directly related to its rotational correlation time. Binding of macromolecules increases anisotropy, allowing equilibrium determinations of sequential protein interactions⁹⁻¹¹. Our studies used a fluorescein-labelled somatostatin CRE and 30 nM CREB. Under the

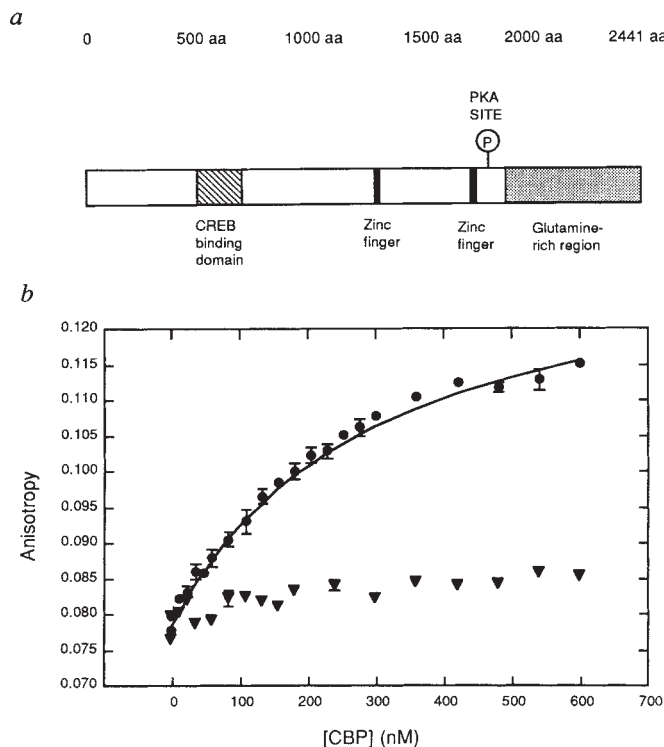


FIG. 1 Binding of phosphoCREB to CBP. *a*, Schematic representation of the CBP molecule, showing the CREB binding domain, zinc-fingers, PKA phosphorylation site and glutamine-rich region. *b*, Fluorescence anisotropy measurements of the CBP:phosphoCREB:CRE interaction. Circles represent measurements using phosphoCREB, triangles represent measurements using non-phosphorylated CREB (mean anisotropy $\pm$ s.d.). Data are fit to a simple non-cooperative model of CBP interaction shown by the solid line. The apparent dissociation constant for the interaction between phosphoCREB and GST-CBP, determined by averaging three independent experiments, is 2.2×10^{-7} M.

METHODS. A 23 base 5'-fluoresceinated oligodeoxyribonucleotide corresponding to the sense strand of the somatostatin CRE (5'-F-CCTTGGCTGACGTGACAGAGAGC-3'; obtained from Genosys Inc.) was annealed to an oligonucleotide (5'-GCTCTCTCTGACGTGACCAAGG-3') corresponding to the CRE antisense strand to form a duplex, F-CRE. No single-stranded DNA was detectable in the annealed F-CRE preparation, as judged by native PAGE (not shown). GST-CBP was produced in *Escherichia coli* DH5 α by inserting a *NcoI*-*Bam*HI fragment of the human CBP cDNA (encoding amino acids 117-661) into pGEX-KG and purifying the fusion protein as described¹⁹. CREB and phosphoCREB were also produced as described⁷. Fluorescence polarization measurements used an SLM Instruments Model 8000 spectrofluorimeter. Samples were excited at 490 nm and emission was measured using 515-nm cutoff filters. Binding reactions (1 ml) contained 25 mM Tris-HCl, pH 7.6, 50 mM NaCl, 5 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 5% glycerol, 10 nM F-CRE, with 30 nM CREB or phosphoCREB and 0-600 nM GST-CBP. Samples were incubated for 2 min at 20 °C between protein additions. Under these conditions, the maximum change in anisotropy occurs within 15-30 s. Four measurements were done at each titration point, with 10-s integration times for each measurement. Total volume of the reactions increased by less than 10% during each experiment. Binding constants for the CBP-phosphoCREB interaction were obtained by fitting the data to a hyperbolic function by nonlinear least squares analysis. We were unable to detect significant binding of GST alone to the phosphoCREB-CRE complex at concentrations up to 2 μ M (data not shown).

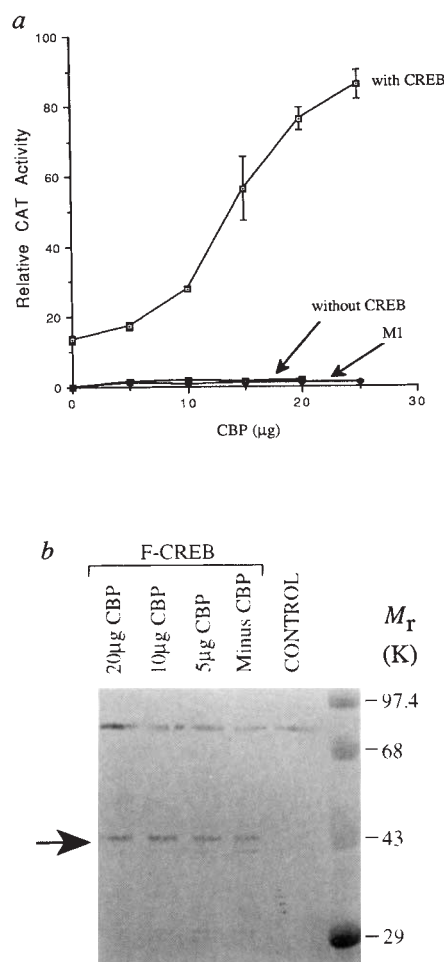


FIG. 2 CBP potentiates CREB activated gene expression *in vivo*. *a*, F9 teratocarcinoma cells were transiently transfected with various mixtures of p(-71)SRIF-CAT³, pRSV-luciferase, pRc/RSV-PKA (encoding the catalytic subunit of PKA; gift from R. Maurer), pRc/RSV-CREB341¹⁴ (an expression vector encoding CREB341), pRc/RSV-CREB341-M1¹⁴ (a CREB expression vector containing a serine to alanine mutation in the PKA phosphorylation site) and pRc/RSV-CBP (an expression vector encoding full-length mouse CBP). The results are expressed as the relative CAT activity (mean $\pm$ s.e., $N > 5$) compared to the CAT activity in the absence of CREB and CBP. CAT activity values are normalized for luciferase activity. *b*, Western blot analysis of cells transfected with p(-71)SRIF-CAT, pRSV-luciferase, pRc/RSV-PKA, pRc/RSV-CBP and pRc/RSV-FLAG-CREB341 (F-CREB; an expression vector encoding CREB341 tagged at the N-terminus with a FLAG epitope). Control cells received no exogenous F-CREB or CBP, other cells received F-CREB and various amounts of CBP. M_r markers are indicated on the right.

METHODS. *a*, F9 cells were seeded at 3.5×10^5 cells per plate 1 day before transfection with 5 μ g p(-71)SRIF-CAT, 2 μ g RSV-luciferase, 4 μ g pRc/RSV-PKA, 4 μ g pRc/RSV-CREB341 or pRc/RSV-CREB341-M1, and 0, 5, 10, 15, 20 or 25 μ g pRc/RSV-CBP as described previously¹⁴. pRc/RSV was used to normalize the amount of DNA to 40 μ g for each condition. CAT and luciferase activities were assayed as described¹⁴. *b*, Cells were transfected as above using 4 μ g pRc/RSV-FLAG-CREB341 and 0, 5, 10 or 20 μ g of pRc/RSV-CBP. Western blot analysis used the anti-FLAG monoclonal antibody M2 as described by the manufacturer, IBI. Equal amounts of protein were loaded onto each lane.

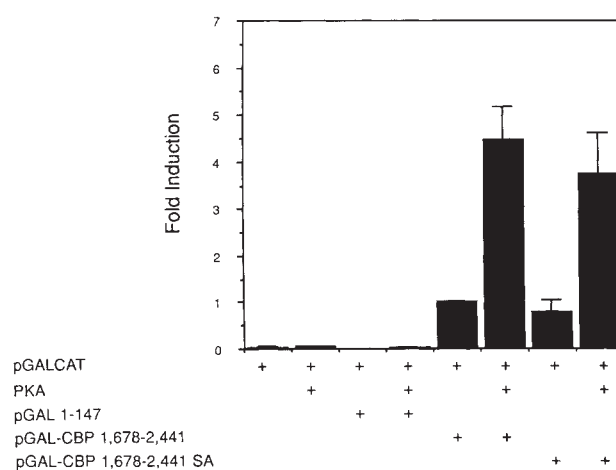
experimental conditions used, the CRE is fully saturated by CREB (data not shown). Specific, saturable binding of CBP to the phosphoCREB:CRE complex was demonstrated by the increase in fluorescence anisotropy (Fig. 1b). The dissociation constant for this interaction was 2.2×10^{-7} M. CBP did not interact with non-phosphorylated CREB, confirming our initial results⁷. Additionally, CBP did not affect the ability of CREB or phosphoCREB to bind to the somatostatin CRE (data not shown). The affinity of CBP for phosphoCREB was similar to that of other physiologically important protein:protein interactions¹¹, but considerably less than that between CREB and the CRE¹². This suggests that CREB may bind constitutively to the CRE, and that the phosphorylation-induced change in affinity of CREB for CBP may be a critical regulatory step for transcriptional activation.

In most cells, the generation of cAMP is the rate-limiting step for the transcriptional activation of CRE-containing genes.

CREB appears to be limiting in F9 teratocarcinoma cells, because exogenous CREB is required to mediate expression of CRE-reporter genes^{13,15}. We hypothesized that in the presence of exogenous CREB and PKA, subsequent components of the transcriptional machinery should become limiting. We asked, therefore, whether vectors encoding CREB and CBP could coordinately stimulate expression of a CRE reporter in the F9 cell model (Fig. 2a). In the absence of exogenous CBP, the combination of CREB and PKA activated expression of the reporter by about 15-fold, as reported previously^{13,15}. In the presence of exogenous CREB and PKA, CBP increased transcription in a dose-dependent manner up to a level of about 90-fold. CBP did not activate gene expression in the absence of CREB, even in the presence of PKA, and CREB mutated at the PKA phosphorylation site did not allow CBP to activate expression. CREB levels were not altered by the addition of exogenous CBP (Fig. 2b) and, in the absence of PKA, CREB did not permit CBP-

FIG. 3 The transcriptional activation domain of CBP resides within its C terminus. PC12 cells were transfected with 5 μ g of the reporter pGAL-CAT and equimolar amounts of the following plasmids as indicated; pGAL-CBP 1,678–2,441 (an RSV expression plasmid encoding the DNA-binding domain of GAL4 fused to amino acids 1,678–2,441 of CBP); pGAL-CBP 1,678–2,441 SA (identical to pGAL-CBP 1,667–2,441 except serine 1,782 of CBP is mutated to alanine, thereby abolishing the consensus site for phosphorylation by PKA); and pGAL 1–147 (an expression plasmid encoding the DNA binding domain of GAL4). Where indicated, 5 μ g of pRc/RSV-PKA was also included. Transcriptional activity is reported as fold-induction compared to the basal activity of pGAL-CBP 1,678–2,441. Mean $\pm$ s.e. are shown; >5 experiments were done for each set of conditions.

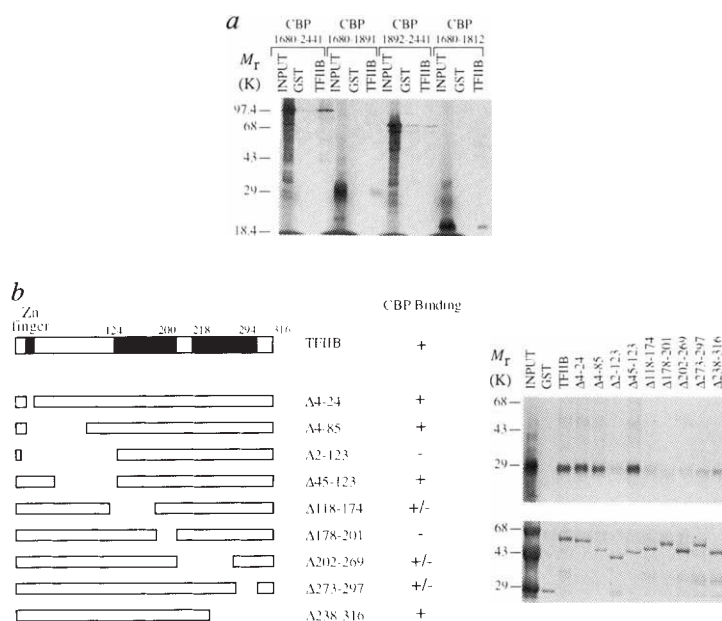
METHODS. The plasmid pGAL-CBP 1,678–2,441 was constructed by subcloning the 3' EcoRI fragment of the mouse CBP cDNA into the EcoRI site of the plasmid pGem7. This fragment was subsequently removed by digestion with XbaI and BamHI and subcloned into XbaI- and BamHI-digested pGAL 1–147 (gift from M. Gilman). Serine 1,772 was mutated to alanine by site-directed mutagenesis using an Altered Sites kit from Promega and changes were confirmed by nucleotide sequencing. The pGAL-CAT reporter was the pGal4/Elb TATA plasmid described in ref. 20. All transfections were normalized for the amount of DNA and RSV promoter by the addition of pRc/RSV (Invitrogen) and calf thymus carrier



DNA (BRL). CAT activity was assayed as described¹⁴ and values were normalized to the amount of protein used in each assay.

FIG. 4 The C terminus of CBP binds TFIIB. *a*, A GST/full-length TFIIB fusion protein¹⁶ was immobilized on glutathione-agarose beads and incubated with *in vitro* translated, ³⁵S-labelled fragments of CBP. CBP fragments used in each binding reaction are indicated at the top of the figure, input refers to one-fifth of *in vitro* translation products used in the binding assays, GST shows binding to GST alone, and TFIIB shows binding to a GST/full-length TFIIB fusion protein. *M_r* markers are shown on the left side of figure. *b*, Deletion mutants of TFIIB fused to GST are shown on the left, as described in ref. 16. Right side of figure shows binding of *in vitro* translated, ³⁵S-labelled CBP 1,680–1,891 to the various GST-TFIIB deletion mutants (top) and Coomassie blue staining of the GST-TFIIB fragments to control for protein binding to the agarose beads (bottom).

METHODS. Complementary DNA fragments corresponding to CBP 1,680–2,441, 1,680–1,891, 1,892–2,441 and a PCR product corresponding to CBP 1,680–1,812 were cloned into pET23b (Novagen). ³⁵S-labelled CBP was produced in a TNT-coupled reticulocyte system as described by the manufacturer (Promega). Labelled proteins were preincubated with glutathione-agarose beads in binding buffer (100 mM NaCl, 1 mM EDTA, 20 mM Tris-HCl, pH 8.0, 0.5% NP-40) for 1 h at room temperature. Roughly equimolar amounts of GST, GST-TFIIB, or GST-TFIIB deletion mutants were coupled to glutathione-agarose beads in 0.5 ml binding buffer for 1 h at room temperature before addition of labelled proteins. Labelled CBP fragments were incubated with GST fusion proteins for 1 h at room temperature, washed, and the bound



fragments were eluted with SDS-PAGE loading buffer and separated on 10% SDS-polyacrylamide gels. Labelled products were detected by autoradiography.

mediated gene induction (data not shown). The finding that CBP induces gene expression only if CREB and PKA are also added implicates CBP in the pathway responsible for cAMP-activated gene transcription.

To determine what portion of CBP allowed transactivation, we examined a fusion gene containing residues 1,678–2,441 of CBP fused to the DNA-binding domain of GAL4. This region of CBP contains the second putative zinc-finger motif, the consensus PKA phosphorylation site, and the glutamine-rich region (Fig. 1a). pGAL-CBP (1,678–2,441) was more than 100-fold more active than pGAL 1–147 in inducing expression of a GAL-CAT reporter (Fig. 3), and was thus considerably more active than the full-length GAL-CBP gene⁷. Whether this difference is due to the removal of an autoinhibitory domain or to more efficient production of the smaller CBP fragment is unknown. We had previously found that PKA increased activity of the full-length GAL-CBP by four- to five-fold⁷, and a similar level of induction by PKA was seen using the smaller GAL-CBP gene. However, mutating the consensus PKA site in CBP did not affect either basal or PKA-stimulated expression of the reporter. Thus, although the interaction of phosphoCREB with CBP appears to be the key regulatory step in PKA-activated transcription, indirect effects of PKA may influence CBP function as well.

Having determined that the C-terminal portion of CBP allowed transcriptional activation, we asked whether this region interacts with basal transcription factors. In particular, the finding that VP16 interacts with TFIIB¹⁶ led us to consider whether CBP makes similar contacts. This hypothesis was supported by evidence that the yeast coactivator ADA-2, which shares a 50 amino-acid zinc-finger motif with CBP^{7,8}, displays genetic interactions with the yeast TFIIB (L. Guarante, personal communication).

Binding of CBP to TFIIB was tested by using GST-TFIIB fusion proteins linked to glutathione agarose beads (Fig. 4a). Specific binding was observed with the 1,680–2,441 fragment of CBP, although a small amount of nonspecific binding to GST alone was also detected. When the C-terminal region of CBP was separated into fragments extending from 1,680–1,891 and from 1,892–2,441, specific binding was detected only with the former portion. A smaller fragment, encompassing only residues 1,680–1,812, also bound specifically. This 133 amino-acid region of CBP contains the zinc-finger structure shared with ADA-2.

Binding between CBP and deletion mutants of TFIIB was examined by using the CBP 1,680–1,891 fragment. These studies suggested that CBP may require an amino-terminal region of TFIIB in addition to the two repeats for binding (Fig. 4b). Previous work indicated that VP16 binding requires only the two TFIIB repeats¹⁶. Thus, CBP and VP16 interact with overlapping but also distinct portions of TFIIB.

Our data support the model that CBP serves as a coactivator for the phosphorylated form of CREB. The interaction of the zinc-finger region of CBP with TFIIB is intriguing given the conservation of this sequence with ADA-2, but does not rule out interactions with other basal transcription factors¹⁷ or even direct interactions between basal factors and portions of CREB¹⁸. □

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Activation of cAMP and mitogen responsive genes relies on a common nuclear factor

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A NUMBER of signalling pathways stimulate transcription of target genes through nuclear factors whose activities are primarily regulated by phosphorylation. Cyclic AMP regulates the expression of numerous genes, for example, through the protein kinase-A (PKA)-mediated phosphorylation of transcription factor CREB at Ser 133^{1,2}. Although phosphorylation may stimulate transcriptional activators by modulating their nuclear transport or DNA-binding affinity³, CREB belongs to a class of proteins whose phosphorylation appears specifically to enhance their *trans*-activation potential^{1,2,4}. Recent work describing a phospho-CREB binding protein (CBP)⁵ which interacts specifically with the CREB *trans*-activation domain prompted us to examine whether CBP is necessary for cAMP regulated transcription. We report here that microinjection of an anti-CBP antiserum into fibroblasts can inhibit transcription from a cAMP responsive promoter. Surprisingly, CBP also cooperates with upstream activators such as c-Jun, which are involved in mitogen responsive transcription⁶. We propose that CBP is recruited to the promoter through interaction with certain phosphorylated factors, and that CBP may thus play a critical role in the transmission of inductive signals from cell surface receptor to the transcriptional apparatus.

To characterize the functional properties of CBP, we developed an antiserum directed against amino acids 634–648 within the CREB binding domain of CBP. Using this antiserum in western blot assays, we detected a 265K polypeptide (Fig. 1a, lane 1), as predicted from the complementary DNA⁵, which coincided with the predominant phospho-CREB binding activity in HeLa (Fig. 1a, lane 2) and NIH-3T3 (not shown) nuclear extracts by 'far-western' blot assay. The 265K phospho-CREB binding activity appeared to be specific for Ser 133-phosphorylated CREB because no such band was detected with CREB labelled to the same specific activity at a non-regulatory phospho-acceptor site (Ser 156) by casein kinase II (CKII)⁷ (Fig. 1b). To determine whether the major phospho-CREB binding protein in HeLa and 3T3 cells corresponds to the CBP immunoreactive protein, we prepared immunoprecipitates from crude nuclear extracts using the CBP antiserum. Far-western analysis

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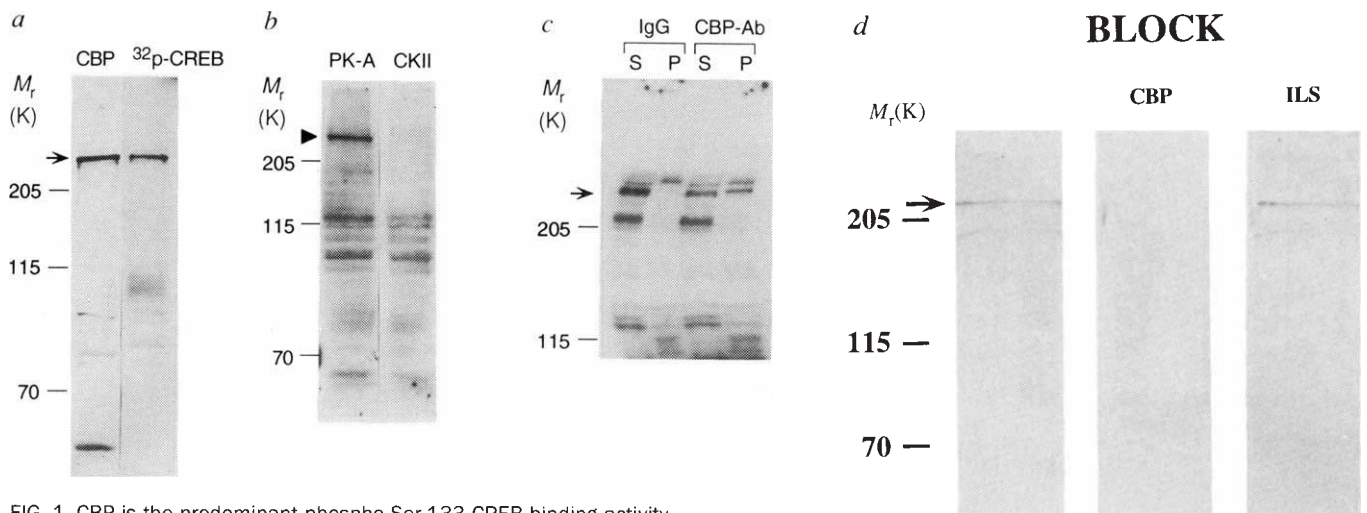


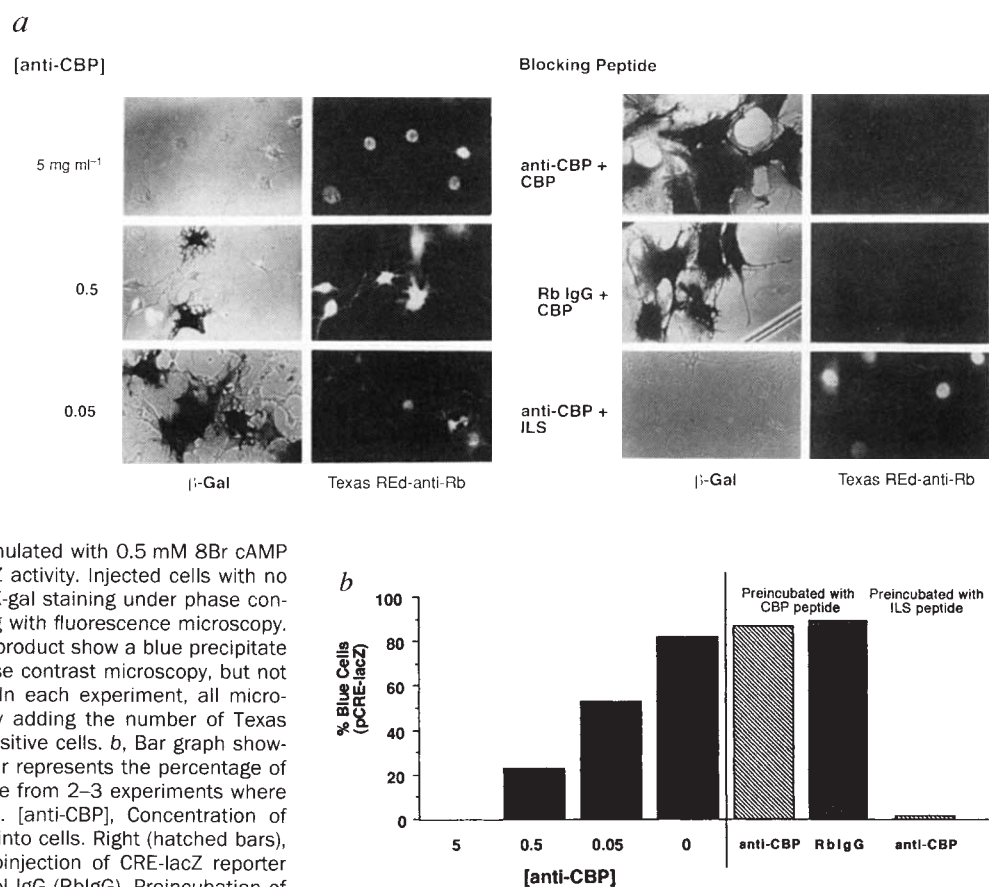
FIG. 1 CBP is the predominant phospho-Ser 133 CREB-binding activity in nuclear extracts. *a*, Western (CBP) and far-western (^{32}P -CREB) blot analysis of partially purified CBP protein from HeLa nuclear extract following SDS-PAGE and transfer to nitrocellulose. Additional immunoreactive bands represent proteolytic fragments of CBP. *b*, Far-western blot of crude HeLa nuclear extract using ^{32}P -labelled CREB phosphorylated with PKA or casein kinase II (CKII). Arrow (*a*, *b* and *c*) points to major 265K band. *c*, Far western blot analysis of immunoprecipitates prepared from HeLa nuclear extracts with control IgG (IgG) or affinity-purified CBP antiserum (CBP-Ab). CREB binding activity was detected with ^{32}P -labelled CREB phosphorylated with PKA. S,P, supernatant and pellet fractions respectively. *d*, Western blot analysis of whole cell extracts from NIH-3T3 cells using CBP antiserum blocked with CBP peptide

(CBP), ILS peptide (ILS), or control (-). Arrow, immunoreactive CBP band. Lighter band at 180K corresponds to proteolytic fragment of CBP. Faint higher M_r bands are also detectable with second anti-CBP antiserum against an N-terminal CBP peptide⁵, and may represent alternatively spliced or otherwise modified form of CBP.

METHODS. Rabbit CBP antiserum was developed against a synthetic peptide of CBP extending over amino acids 634–648 (KVEGDMYES-ANSRDE). Crude antiserum was affinity purified on a synthetic CBP peptide column¹⁸. Far-western and western blot assays were as described previously^{4,5}.

FIG. 2 CBP is required for cAMP-inducible transcription. *a*, Left, Representative fields of NIH-3T3 cells injected with pCRE-lacZ reporter plasmid plus 5, 0.5 and 0.05 mg ml⁻¹ affinity-purified CBP antiserum. Total antibody concentration in micro-injected cells was maintained at 5 mg ml⁻¹ by adjusting with control rabbit IgG. Injected cells were stimulated with 0.5 mM 8Br-cAMP plus IBMX for 4 h, then fixed and assayed for lacZ activity (β -Gal) as well as antibody content (Texas red anti-Rb). Right, Effect of CBP antiserum on CRE-lacZ reporter activity following pre-treatment of CBP antiserum with synthetic CBP peptide (anti-CBP+CBP) or unrelated peptide (anti-CBP+ILS). (Rb IgG+CBP), Control rabbit IgG pre-treated with CBP peptide. NIH-3T3 cells were injected with CRE-lacZ reporter plus various CBP antisera, stimulated with 0.5 mM 8Br cAMP plus IBMX for 4 h, and assayed for lacZ activity. Injected cells with no lacZ reporter activity show white after X-gal staining under phase contrast microscopy and Texas red staining with fluorescence microscopy. Injected cells expressing the lacZ gene product show a blue precipitate after X-gal staining as detected by phase contrast microscopy, but not Texas red staining due to quenching. In each experiment, all micro-injected cells can be accounted for by adding the number of Texas red positive plus the number of lacZ positive cells. *b*, Bar graph showing summary of injections in *a*. Each bar represents the percentage of positive cells expressing β -galactosidase from 2–3 experiments where 100–200 cells were injected in each. [anti-CBP], Concentration of affinity-purified CBP antiserum injected into cells. Right (hatched bars), Per cent lacZ-positive cells after microinjection of CRE-lacZ reporter with CBP antiserum (anti-CBP) or control IgG (RbIgG). Preincubation of antiserum with CBP peptide or nonspecific ILS peptide (1 mg ml⁻¹) as indicated.

METHODS. NIH-3T3 cells were cultured in 5% CO₂ atmosphere in Dulbecco's MEM supplemented with 10% fetal calf serum. Forty eight hours



before injection, cells were passaged onto scored glass coverslips and made quiescent by incubation in medium containing 0.05% fetal calf serum for 24 h^{7,19}.

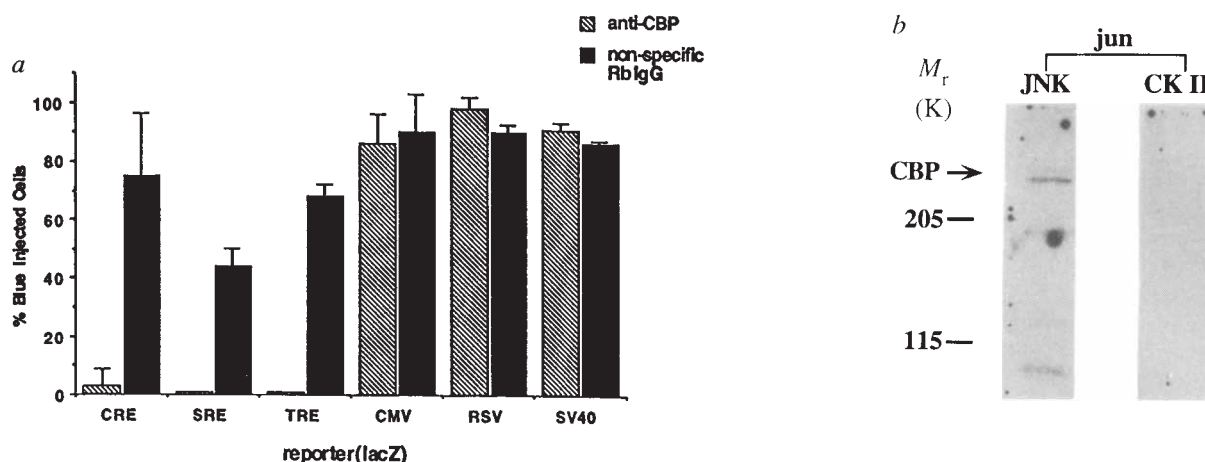


FIG. 3 Multiple signalling pathways use CBP. **a**, Bar graph showing summary of CBP antisera injections after 4 h treatment with either 0.5 mM 8Br-cAMP plus IBMX (CRE), 20% fetal calf serum (SRE), or 200 ng ml⁻¹ TPA (TRE). Bars represent percentage of lacZ positive (blue) cells (mean $\pm$ s.d.) from 3–5 experiments where 100–200 cells were injected in each. Injected cells were identified by immunofluorescence and/or lacZ staining. Reporter (lacZ), reporter plasmid microinjected into 3T3 cells. CRE, SRE, TRE lacZ reporter activities were determined after microinjected cells were treated as described above. CMV, RSV and SV40 lacZ reporter activities were measured in the absence of inducers. Hatched bars indicate per cent blue cells after microinjection with CBP antiserum. Solid bars indicate per cent blue

cells following injection with control rabbit IgG. **b**, JNK-phosphorylated c-Jun binds to CBP *in vitro*. Far western analysis of crude HeLa nuclear extracts using ³²P-labelled recombinant Jun protein phosphorylated to the same specific activity *in vitro* with either Jun-kinase (JNK)⁹ or casein kinase II (CK II). Arrow points to band corresponding to 265K CBP protein.

METHODS. CRE, TRE and SRE lacZ reporter plasmids were constructed as previously described²¹ using a minimal RSV promoter, into which was inserted five consensus CRE sites from the vasoactive intestinal peptide promoter (CRE-lacZ), 3 tandem TRE elements from the rat collagenase promoter (TRE-lacZ) or 5 copies of the SRE sequence from the human c-fos promoter (SRE-lacZ).

of these fractions (Fig. 1c) revealed a 265K band in samples incubated with CBP antiserum but not with control IgG.

To examine whether the phosphorylation-dependent interaction between CREB and CBP was critical for cAMP responsive transcription, we used a microinjection assay using CBP antiserum which also recognized a 265K protein in whole-cell extracts of NIH-3T3 cells (Fig. 1d). After microinjection into nuclei of NIH-3T3 cells, a CRE-lacZ reporter was markedly induced by treatment with 8-Br cAMP plus IBMX. Coinjection of CBP antiserum with the CRE-lacZ plasmid completely inhibited cAMP-dependent activity in a dosage-dependent manner (Fig. 2a, b left), but control IgG had no effect on this response (Figs 2a, b right and 3). To determine whether the inhibitory effect of CBP antiserum was dependent on antibodies which reacted specifically with CBP, we did peptide blocking experiments (Fig. 2a, b right). CBP antiserum, pre-incubated with synthetic CBP peptide, could not recognize the 265K CBP product on a western blot of whole NIH-3T3 cell extracts (Fig. 1d) and, correspondingly, could not inhibit CRE-lacZ reporter activity on microinjection into NIH-3T3 cells (Fig. 2a, b right). But antiserum treated with an unrelated synthetic peptide (ILS) showed full activity in both western and microinjection assays, suggesting that the ability of the antiserum to bind CBP was critical for its inhibitory effect on cAMP-dependent transcription. These results were further supported by transfection assays in COS-7 cells, in which a CBP expression vector was found to potentiate the PKA-dependent activity of wild-type but not a phosphorylation-defective (Ser 133 to Ala 133) mutant CREB protein 2.5-fold (data not shown).

Previous reports showing that serum and phorbol esters stimulate their target genes through phosphorylation-dependent *trans*-activators^{8–13}, led us to examine whether CBP might also function in these signalling pathways (Fig. 3a). Whereas serum and TPA could stimulate reporter activity in NIH-3T3 cells microinjected with either serum responsive element (SRE)-lacZ or TPA-responsive element (TRE)-lacZ indicator plasmids, respectively, coinjected CBP antiserum completely blocked both responses. By contrast, CBP antiserum had no effect on basal

expression from any of the viral promoter constructs we tested, including cytomegalovirus (CMV-lacZ), Rous sarcoma virus (RSV-lacZ), and SV40 (SV40-lacZ) (Fig. 3a). These results indicate that CBP can indeed discriminate between basal- and signal-dependent activities *in vivo*.

Like CREB, c-Jun transcriptional activity is stimulated by phosphorylation of one major site in its activation domain, Ser 73 (ref. 11 and T.S. *et al.*, manuscript in preparation). Indeed, the protein kinases involved in c-Jun phosphorylation and transcriptional activation, JNK 1 and 2, are also induced by various mitogens^{9,14} prompting us to examine whether phosphorylation of c-Jun allows it to bind CBP. Using ³²P-labelled recombinant c-Jun protein, phosphorylated at Ser 63 and Ser 73 with JNK, we did far-western blot assays on crude HeLa nuclear extracts (Fig. 3b). JNK-phosphorylated c-Jun protein could bind CBP with comparable affinity to CREB. Casein kinase II (CKII) also phosphorylates c-Jun at Thr 231, Ser 243, and Ser 249¹⁵. Unlike JNK, however, CKII appears specifically to inhibit c-Jun DNA binding activity without modulating its *trans*-activation potential¹⁵. Consistently, CKII-phosphorylated c-Jun could not bind to CBP (Fig. 3b), suggesting that interaction between CBP and c-Jun requires phosphorylation of the transcriptionally stimulatory sites Ser 73 and Ser 63. In transfection assays, a CBP expression vector could augment activity from a cotransfected plasmid expressing wild-type but not phosphorylation-defective (Ala 63/73) c-Jun 3.5-fold in F9 teratocarcinoma cells (not shown).

CBP shares extensive sequence identity with the E1A-associated protein p300, particularly in regions that bind phospho-CREB (86% identity, amino acids 580–680) and E1A (84% identity, amino acids 1,000–1,880)¹⁶. Indeed, the ability of E1A selectively to inhibit transcription from certain promoters through P300 suggests that E1A may interfere with the ability of CBP or P300 to function as a coactivator. In this regard, overexpression of CBP did not appear to potentiate c-Jun and CREB activities in all cell lines, possibly reflecting a requirement for additional CBP-associated proteins. The recruitment model which we propose for CBP predicts that two proteins which

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interact with CBP, such as CREB and c-Jun, may interfere with or 'squench' one another when intracellular levels of CBP are limiting. Competition for a common coactivator like CBP may in part explain the cross-talk which is commonly observed between disparate signalling pathways¹⁷. □

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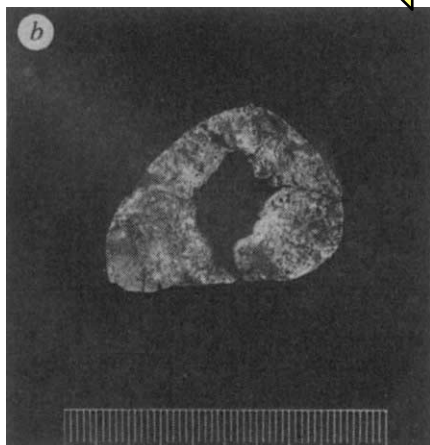
ERRATUM

A hominid tibia from Middle Pleistocene sediments at Boxgrove, UK

M. B. Roberts, C. B. Stringer & S. A. Parfitt

Nature **369**, 311–313 (1994)

BECAUSE of a reprographic error, the cross-section of the tibia shown in Fig. 3b was printed with the wrong scale. The correct version is shown below. Scale bar, 10 cm.



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Serum-free cell culture system for the long-term culture of differentiated hepatocytes.

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p53 transcriptionally activates a number of genes including the WAF1/CIP1 gene. The p21^{WAF1} protein is a potent inhibitor of cyclin-dependent kinases (cdk) active in the G1 phase of the cell cycle. Interaction between a G1 cyclin and a cdk is necessary for

full kinase activity. The active cyclin/cdk complex is required for cells to enter S phase of the cell cycle. In response to DNA damaging agents, p53 may arrest cells by activation of the WAF1 gene thus inhibiting the cyclin/cdk activity. Oncogene Science's new WAF1 (Ab-1) **mouse monoclonal antibody detects p21^{WAF1}** by immunoblotting and immunoprecipitation (Reader Service No. 104).

Dako's new histone H3 probe is an S-phase specific, hapten-labelled probe that can be used as a **cytoplasmic marker of proliferating cells** in routinely processed tissue utilizing *in situ* hybridization (Reader Service No. 105). The long single-stranded probe design is optimal for mRNA hybridization and signal generation. Dako says that, because it is a cytoplasmic marker, it is suitable for double-staining procedures with nuclear antigens. When used in conjunction with the company's ISH detection system, results can be obtained in 5 h, according to Dako. The chromogen used is BCIP/NBT.

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Natural source phospholipids.

able include high-purity L- α -phosphatidylcholines, L- α -phosphatidylethanolamines, L- α -phosphatidylglycerol, L- α -phosphatidylinositol, platelet activating factor and cardiolipin from various natural sources.

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Chambers for chemotaxis.

25 μ l and can be used with either polycarbonate or nitrocellulose filters. With the 96-well format, once removed, filters can be loaded directly into plate readers. Other options include 10- and 12-well chambers and a three-tiered configuration that is suitable for the observation of cells using an inverted microscope.

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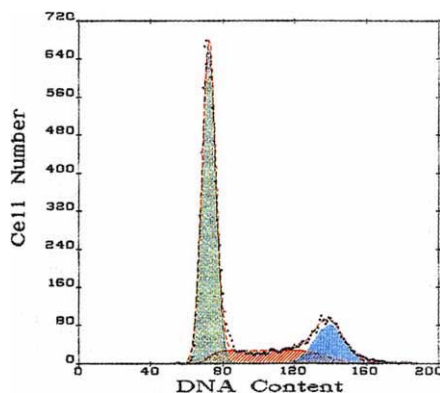
MAP alkaline phosphatase, available from ProZyme for research and diagnostic applications, is intended as a replacement for calf intestinal alkaline phosphatase in enzyme labelling applications where lower nonspecific binding, higher temperature stability and lower sera interaction are required (*Reader Service No. 108*). It lacks post-translational modifications such as the glycosyl and glycolipid moieties present on the calf intestinal enzyme, thought to be responsible for that enzyme's nonspecific interactions. MAP has a stated half-life of about 4 h at 80 °C. MAP is also available as a streptavidin conjugate for those researchers using biotin-binding systems.

To avoid the presence of undesired proteases during isolation and purification of intact peptides and proteins, which can lead to reduced yields of pure and active proteins particularly for proteins isolated from cellular extracts, ICN Biomedicals recommends its new range of **protease inhibitors** (*Reader Service No. 109*). Included among these are AEBSF, aprotinin, leupeptin, pepstatin A and the new protease inhibitor cocktail kit (sufficient to prepare 100 ml of cocktail).

■ Molecular means

Ambion's CAT-Direct kit provides a means for a **direct determination of CAT-linked promoter activity** by ribonuclease protection and should be of interest to researchers that use reporter gene expression systems for the study of transcriptional regulation (*Reader Service No. 110*). The ribonuclease method eliminates variables which are not linked to transcription such as translational efficiency and protein (enzyme) turnover. Ambion says that this is especially useful when CAT transcription is down regulated as the long half-life of the CAT protein makes it difficult to detect rapid decrease in the level of CAT transcript. The kit can also be used to detect CAT RNA expression in all eukaryotic and prokaryotic expression systems.

Exalpha's new NuCycl DNA kits should be of use in flow cytometry, image analysis or fluorescent microscopy (*Reader Service No. 111*). The NuCycl PI and DAPI kits may be used to prepare nuclei from cells or tissues for DNA flow cytometry; Nucl Trout DNA Standard supplies DNA reference values, necessary in human ploidy studies. The

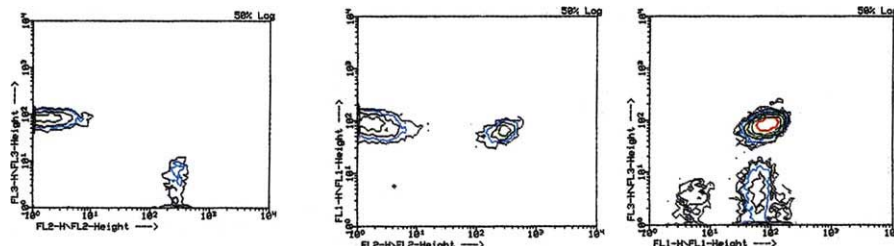


Human T-cell line prepared and stained with Exalpha's NuCycl PI.

NuParaffin paraffin extraction kit may be used to isolate single cells and nuclei from paraffin, ethanol and other fixed specimens for DNA analysis or cell surface staining. Exalpha also offers Alpha3 — a three-colour system for flow cytometry utilizing the company's new colour, AlphaRed. Alpha3 reagents now available are CD3/CD4/CD8, CD3/CD4/CD25, CD3/CD8/CD25, negative controls and AlphaRed conjugated to streptavidin.

Raggio-Italgene has launched a new diagnostic kit, the C-Trak CF ΔF 508, which **detects the presence of the ΔF 508 mutation in cystic fibrosis patients and carriers** (*Reader Service No. 112*). Later this year, the company plans to offer an improved version of the kit, the C-Trak CF Plus, which, in addition to detecting the presence of the ΔF 508 three-base-pair deletion, will simultaneously detect five of the mutations most frequently encountered in the western world with a stated prediction level of 85–90 per cent. Both kits (which do not require radioactive materials) are designed to be fast; detection after amplification is said to take about an hour.

The AG-9600 AmpliSensor system from Biontronics is designed to streamline **quantitative PCR** by allowing amplification and detection to be carried out in one tube (*Reader Service No. 113*). The system directly monitors the amplified product of up to 96 different DNA or RNA samples in a few seconds, according to the company. Protocols can be adapted using the accompanying universal coupling kit. A Windows-based ASAP program reports the quantitative PCR result in both table and graph formats.

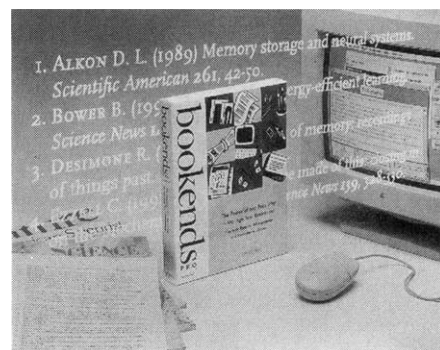


Peripheral blood lymphocytes stained with Exalpha's Alpha3 CD3 FITC-CD8 PE-CD4 AlphaRed. No compensation between FL2 and FL3.

Moreover, the analyser comes with an automated reagent dispensing capability, designed to eliminate the tedium and error associated with multiple PCR pipetting.

■ Software

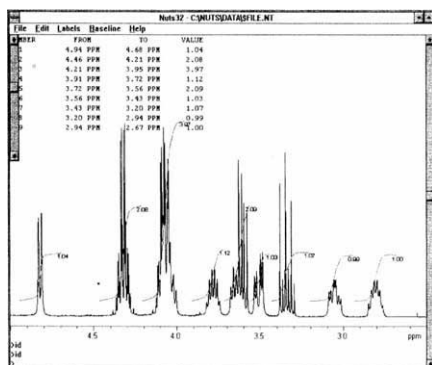
Bookends Pro is a **bibliography management system** for scientists and other professionals who need to track and cite reference information for research and publication purposes (*Reader Service No. 114*). Designed for the Macintosh, Bookends Pro offers a means of collecting, managing and formatting references, citations and quotes, and automatically generates bibliographies and footnotes that conform to publication specifications. Users can import reference information from a variety of online database services including Medline, Dialog, BRS and others, as well as from CD-ROMs



Bookends Pro — bibliography management software for the Mac.

and tab delimited text files. Over 90 predefined formats for generating references and footnotes are included; users may also customize their own. Bookends Pro includes fields that can be customized (up to 30,000 characters) for authors, title, journal, volume, pages, publisher, keywords, notes, abstract and more. Search and find options for locating records include searching on any field or combination of fields, boolean 'and', 'or' and 'not' criteria, unique or relative reference numbers, partial or whole word searches. The program supports System 6.0.5 or later and costs US\$149.

NUTS is Acorn NMR's new Windows-based **NMR processing software package** for the PC — 386 with coprocessor or higher (*Reader Service No. 115*). Both one-dimensional and one-/two-dimensional versions are available. Both versions 'auto-import' data from most commercial spectrometers. The company says that NUTS runs as a 32-bit application, to speed up operations that are heavy on calculations. The 1-D version includes multiple options for most processes, including Fourier transformation, automatic and real-time phasing, baseline correction and apodization. There is a real-time readout of peak information at the cursor position. Integrals and peaks can be labelled on the screen and plots, peak and integral lists can also be printed on the

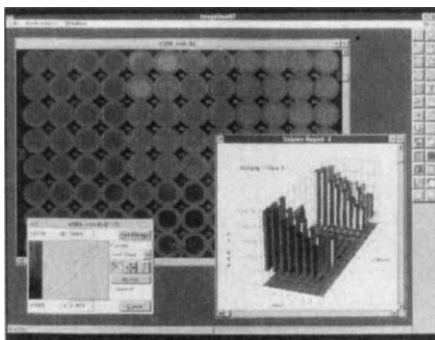


NUTS: a Windows-based NMR processing software package.

screen and plots, as well as saved to the Notepad for insertion into word-processing programs. Spectra may be saved as Windows Metafiles, imported into word-processing programs and sized for inclusion in reports and posters. Spectrum addition/subtraction and dual display features are available, as are a multiple-line deconvolution routine and a 10-spin spectrum simulation program based on chemical shifts and coupling constants. The 2-D version includes all the features of the 1-D version, as well as macros to perform 2-D processing of magnitude, TPPI and States-type hypercomplex data. Quick intensity maps and contour plots may be drawn. 2-D peak picking (with the mouse) on contour plots generates a table of crosspeak information and labels the peaks. The mouse can be used for the real-time display of slices overlaid on contour plots. Projection and stacked plots may be made of an entire data set or of a selected region.

A new upgrade to ImageQuANT, the **system and analysis software** for the Molecular Dynamics line of scanners, is now out (Reader Service No. 116). ImageQuANT 4.1 offers users many new features, including automatic volume integration and push-button reporting. The software contains a complete set of quantitative and qualitative image analysis tools. The tools for viewing images include unlimited image zooming, fast image scrolling, real-time grey/colour

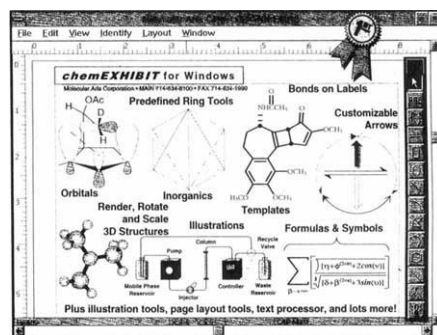
adjustment, and FluorImager system two-colour image support. Quantitative tools include automatic band or spot finding, user-drawn regions of interest, automatic volume integration, and reporting to Microsoft Excel. Lane profiles, including bent or curved lanes, allow users to analyse one-dimensional data. The automatic peak finding and baseline correction features work on multi-



ImageQuANT software for Windows NT.

ple lanes simultaneously and can output data directly to the printer after it has been processed. Requirements include a 486-based computer with at least 16 MB of RAM, Windows NT 3.1 and a high-resolution monitor capable of displaying 256 colours or greys at 800 × 600 resolution, or greater.

A new version of chemEXHIBIT **desktop publishing software for chemical researchers** is now available from Molecular Arts (Reader Service No. 117). Version 3.0 of this Windows-based software program offers documentation, illustration and presentation capabilities designed specifically to meet the needs of chemists. The software allows chemists to display, manipulate and render three-dimensional molecular structures, create two-dimensional (2-D) chemical diagrams, create publication-quality graphic illustrations, import graphic images from third-party applications and document the results of their research. Included in chemEXHIBIT is a full suite of 2-D diagram drawing tools, Chemgram, for creating 2-D pictographs of chemical structures. Chem-

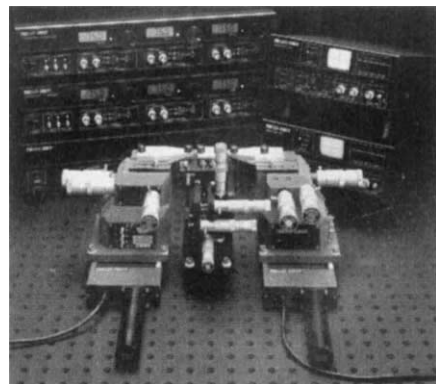


Desktop publishing tool for chemists.

gram comes with 48 diagram drawing tools for drawing bonds, rings, orbitals and atom labels; 'snap to' drawing options; adjustable position, angle and bond length constraints; and automatic superscripts and subscripts in labels. Version 3.0 includes the addition of 40 new or upgraded capabilities including added flexibility in drawing complex lines, curves and arrows, in drawing equations and formulas, in labelling complex diagrams and in handling complex page layouts; in addition to faster operation of the text processor.

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Reader Service No.5

tuators (*Reader Service No. 118*). Applications include launching light into single-mode fibres, characterizing integrated optical devices using end-fire and butt-coupling techniques, and pigtailling of laser diodes and opto-electronic integrated circuits. In addition, the nanopositioners should be of use in patch clamping, a technique used to study the transmission of signals in and between cells.

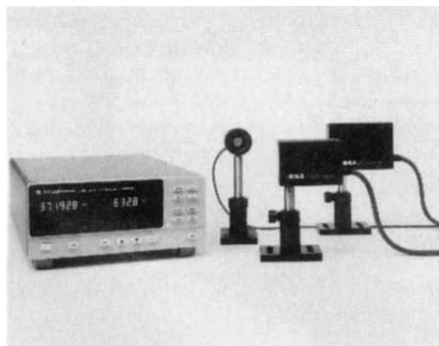
Details of Minitool's new line of **precision micro-tools** for work under the microscope are featured in the company's new catalogue (*Reader Service No. 119*). The full line includes 32 different tool configurations in tip sizes ranging from 0.025 mm to 0.5 mm. Included among these are needles, graters, chisels, spades, forks, mirrors, probers, brushes, spatulas, manipulators, hooks, scribes, burnishers, scrapers, measuring scales and ultra-precision, surgical quality micro-scissors. Also featured are manual and motorized micromanipulators with 0.1 μ m digitally visible resolution control, micro-sharpener sets and precision micro-forceps.



Precision micro-tools.

■ Instrumentation

ILX Lightwave, a supplier of fibre-optic sources/power meters and laser diode products, offers the OMM-6810 **optical multimeter**, which is designed for the simultaneous measurement of laser power and



ILX's laser/fibre optic power/wavemeter operates up to 300 mW.

wavelength and which is available from the distributor AG Electro-Optics (*Reader Service No. 120*). The device can be used to measure laser diodes or outputs direct from fibre-optic systems. Several measurement heads are available; ILX has recently introduced a new OMH-6725 InGaAs head with 3 mW to 300 mW power measurement capability and a 1,000 to 1,600 nm wavelength

resolution range. Wavelength measurement is said to be accurate to ± 0.2 nm. Applications for the new measurement head and multimeter include pump lasers for erbium doped fibre amplifiers where a determination of wavelength is important and output powers (into single mode fibre) are now >70 mW (combined outputs may exceed 200 mW).

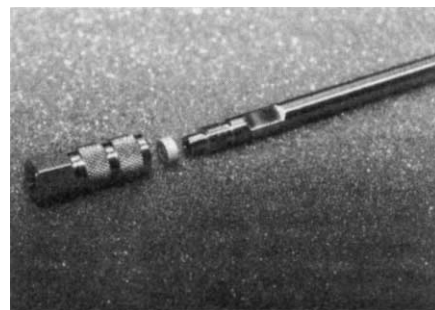
TA Instruments, a supplier of research-grade **thermal analysis equipment**, has introduced a new DSC, the DSC 2010, for researchers on a tight budget (*Reader Service No. 121*). Instrument specifications include high sensitivity (1 μ W) and a temperature range of -180 $^{\circ}$ C to 725 $^{\circ}$ C. The modular make-up allows for future expansion into other thermal analysis techniques. The complete system includes a controller/data analyser, the DSC 2010, an eight-plotter and data analysis software.

By coupling solid-state laser technology with digital signal processing, Protein Solutions has developed the DynaPro-801 **dynamic light scattering molecular size detector**, which is able specifically to measure the hydrodynamic radius and polydispersity of proteins — providing the user with an insight into how proteins behave in solution (*Reader Service No. 122*). A series of application notes — *Screen for Aggregation Prior to Crystallization*, *Monitor Change in Size/Aggregation State as a Function of Varying pH Levels* and *Confirm Protein-Protein Interactions in Solution* — now available from the company, describe how best the dynamic light scattering technology can be applied to protein research.

■ Separation technology

Prosep-Thiosorb-M has been developed by Bioprocessing for the **purification of IgM antibodies** (*Reader Service No. 123*). It is said to combine all the advantages of the rigid Prosep matrix with a stable non-protein, non-carbohydrate ligand. Prosep-Thiosorb-M exhibits selective binding of IgM in the presence of lyotropic (water structuring) salts such as ammonium sulphate or potassium sulphate. It can withstand high flow rates.

Isolation Technologies has introduced a new line of **modular HPLC column systems** designed to provide advantages over



Modular means — see IT.

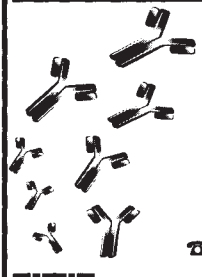
conventional blanks with compression fittings (*Reader Service No. 124*). Each modular system consists of three parts — tube, end fittings and PEEK frit caps. Standard tube sizes include 2.1 mm internal diameter (ID) $\times$ 0.25 inch outer diameter (OD) and 3.0, 3.2, 4.0 and 4.6 mm ID $\times$ 8 mm OD in lengths from 3.3 cm to 30 cm. The IT modular system features tube blanks with its Isobore surface finish, designed to improve column efficiency and performance, especially with 3 μ m and smaller packing materials.

The new VariPerm **hollow-fibre dialysis module** from Bitop is designed to improve conventional FPLC and other liquid chromatography (*Reader Service No. 125*). The device allows continuous, online counter-current dialysis of column eluates before fractionation. Four sizes are available for free-flow electrophoresis, FPLC systems, conventional lab-scale liquid chromatography and large-scale preparative columns. □

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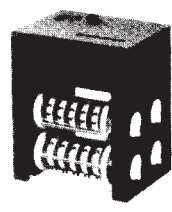
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Reader Service No.12

The scientific labour market in Europe

Josephine Anne Stein and Nicole Kurtz

The United Kingdom, almost alone among European countries, does not have internationalization as an integral part of its education and training policy.

LABOUR market conditions for scientists and engineers in the United Kingdom have been dominated in the past few years by a combination of growth in student numbers and the economic recession. The United Kingdom was the only member state of the European Communities (now the European Union) in which government research and development (R&D) expenditure declined in the second half of the 1980s. Industrial R&D expenditure declined in 1989 and has not grown appreciably since. As trends in hiring research personnel tend to lag behind R&D expenditure, this is consistent with observations that British industry has reduced its recruitment of scientists and engineers. Although there is anecdotal evidence that British employers are tending to hire UK nationals preferentially, unemployment among recent UK graduates appears to be causing more students to stay in higher education, and deterring others from entering science and technology in the first place.

The completion of the Single Market and political steps towards European union have brought the UK system into abrupt contact with the rest of the European Union (EU). International education and training for scientists and engineers has become important to companies seeking to Europeanize their business operations, to higher education institutions preparing students for employment on the mainland of Europe, to public authorities seeking to improve regional and national positions in Europe, and, of course, to scientists and engineers themselves. The United Kingdom supports some schemes for international education and training, but it is the EU that provides the most extensive opportunities for UK scientists and engineers.

EU research fellowships and educational exchange schemes such as Comett and Erasmus have had a substantial impact in the United Kingdom. Nearly a quarter of UK Erasmus students go abroad to study mathematics, the natural sciences and engineering. Both incoming and outgoing students are very enthusiastic about the value of these exchanges, as are most academic staff.

The United Kingdom sent the most students abroad in Comett II, and in receiving students, organizing courses and training projects, was second only to France. Two-thirds of all proposals submitted in 1992 involved a UK partner.

The EU's Human Capital and Mobility (HCM) scheme supports roughly three-quarters of research fellowship exchanges in the Framework Programme. Britain is the most popular destination for HCM individual fellows, with more than a third of the host laboratories in the EU. It also has the greatest number of host laboratories for institutional fellowships and is the member state with the greatest number of participations in networks.

Interestingly, the Office of Science and Technology's recently published *Forward Look* does not refer to the international dimensions of education (other than to acknowledge the research income that universities receive from the EU). Nor does the *Forward Look* address the European dimension of the labour market, or the needs of UK industry for an internationally educated and trained scientific and technological workforce.

UK policy is in striking contrast to other European countries in which internationalization of the science and engineering community is integral to national policy. For example, the Dutch government is committed to internationalizing its entire higher educational system, even for Dutch students enrolled in Dutch universities who will find employment in the Netherlands. France also has an explicit policy to internationalize both its student population and the scientific workforce. Germany and Belgium are implementing educational exchange programmes, primarily on a regional basis. In Ireland and Portugal, EU schemes are integrated into national policies for education and employment of scientists and engineers. Only in Italy is there a comparable focus on reform of the national educational system in response to national demand by employers.

Despite the predominance of national educational systems and labour market conditions in the EU countries, a Europeanized labour market is gradually emerging as a cumulative result of EU and other European programmes for international education and training. International recruitment and employment are no longer unusual. Certain policy issues arise as a consequence.

There are some practical barriers to European educational mobility. Comett, Erasmus and EU research fellowships have suffered poor take-up because of the length of time required to set up exchanges, and mismatches between

academic calendars. There are only five weeks of the year in which universities in all parts of the EU are in teaching session. Information is fragmented and relatively inaccessible, as it arises from so many different quarters and is often aimed at specific groups of people within individual countries or specialized professional occupations.

As exchanges and internationalized curricula develop across Europe, there is no systematic assessment of courses or qualifications. The European Course Transfer System is embryonic and completely voluntary; assessment of equivalences of degrees and diplomas is done on a case-by-case basis. The challenge of developing a comprehensive approach is complicated by the educational reforms under way in a number of European countries. Although a number of so-called 'Euro' degrees are offered by universities or academic/industrial consortia, external recognition is virtually nonexistent.

The Single Market guarantees equal access to education for all European Union nationals and the free movement of labour. Any structural differences in either the educational systems or the national labour markets could therefore lead to imbalances in mobility. This raises the policy question of where the responsibility lies for underwriting the costs of education, which are substantial for science and engineering courses. Should it fall to the home country, the host country or the employer, who may be in yet another country? Should the European Union, given greater responsibilities for education and training under the Maastricht Treaty, assume greater financial responsibility as well?

Although it is not yet clear how far Europeanization of the labour market will progress, there is a clear need for further policy development at both national and European level.

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